ANNUAL REPORT OF PROGRAM ACTIVITIES NATIONAL CANCER INSTITUTE FISCAL YEAR 1974 VOL. IV

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ANNUAL REPORT

OF

PROGRAM ACTIVITIES

NATIONAL CANCER INSTITUTE

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NATIONAL CANCER INSTITUTE

ANNUAL REPORT

DIVISION OF CANCER RESEARCH RESOURCES AND CENTERS

July 1, 1973 through June 30, 1974

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OFFICE OF THE DIRECTOR
DIVISION OF CANCER RESEARCH RESOURCES AND CENTERS

The Division of Cancer Research Resources and Centers, one of four operational divisions of the National Cancer Institute, is responsible for the planning, developing, evaluation and administration of national and international programs of cancer research and education through the awarding of grants.

Organizational Changes and Name Change

The organizational changes of the Division, formalized at the end of fiscal year 1973, are indicated on the accompanying chart. Early in fiscal year 1974, the name of the Division was changed from the Division of Cancer Grants to the Division of Cancer Research Resources and Centers in order to reflect the major program responsibilities of the Division.

Review and Referral Branch

As part of the reorganization, the DCRRC established a Review and Referral Branch to assume responsibility for the review and coordination of all grant applications assigned to the National Cancer Institute. Among its specific functions the Branch:

1. Reviews all NCI-assigned grant applications to determine eligibility and acceptability.

2. Evaluates technical review assignment to determine appropriateness of assigned initial review group.

3. Develops criteria for determining appropriate assignment of applications to specific program area for scientific management and to the NCI initial review groups for technical merit evaluation.

4. Provides assistance and advice to applicants for proper preparation of applications.

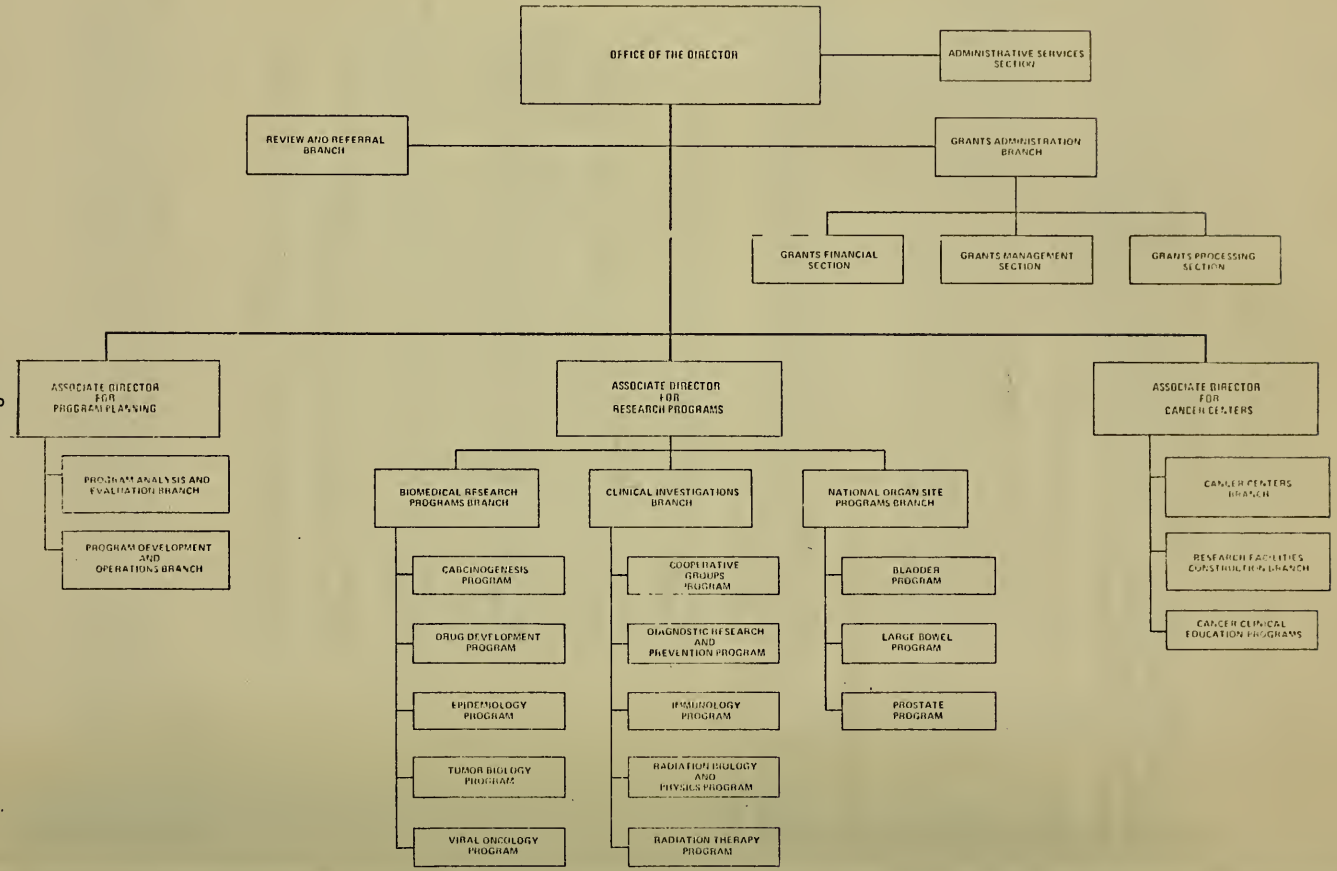
5. Recommends policies and procedures governing technical review of research grant applications.

6. Conducts technical merit review of all applications assigned to the NCI for review.

Budget Review

Grant activities in fiscal year 1974 increased markedly over 1973. Obligations for all grants in 1973 totalled $213 million while the estimate for 1974 is $286 million, an increase of 34%. In addition to the growth in dollars, the number of cancer grant applications increased as indicated below:

DIVISION OF CANCER RESEARCH RESOURCES AND CENTERS



Applications	1971	1972	1973	1974
All Types Reviewed	2,236	2,939	3,373	4,191
Competing Applications Reviewed	1,314	2,002	2,034	2,793
Competing Applications Approved	1,052	1,525	1,369	2,013

FY 1974 BUDGET DISTRIBUTION
(includes Congressional & impounded funds)
(thousands of dollars)

Program	
REGULAR	$ 115,153
Traditional	94,886
Clinical Trials	15,717
Supportive Care	4,000
Scientific Evaluation	550
CENTERS	91,155
Centers	88,536
Exploratory	2,619
CAREERS	2,099
RCDA	1,810
RCA	289
ORGAN SITE	10,003
Bladder	3,420
Large Bowel	3,340
Prostate	3,050
GENERAL RESEARCH SUPPORT GRANTS	776
FELLOWSHIPS	7,085
TRAINING	16,558
Graduate	9,718
Clinical	6,840
CONSTRUCTION	35,900
TOTAL DCRRC	278,729
CANCER CONTROL	7,000
TOTAL	$ 285,729

The budget for fiscal year 1974 went through several phases before it was finally determined. In December 1973, Congress passed and sent to the President the fiscal year 1974 Appropriations Bill providing $551.2 million for the National Cancer Institute; the President had requested $500 million. Included in the bill was a provision allowing the President to reduce appropriations, activities, programs and projects by 5%. Accordingly, under this allocation the NCI received $527.2 million, of which $249.2 million was earmarked for research grants, fellowships, training, and construction. An estimated $7 million is to be awarded in the grants area for Cancer Control.

In addition to the fiscal year 1974 base operating budget, $61.8 million in fiscal year 1973 funds were released to the NCI. Of this amount, $36.5 million was assigned to DCRRC. Specifically, $7.4 million was released for grants (see breakout below). The remaining $29.1 million was reprogrammed into DCRRC from other NCI programs, mainly Cancer Control, and was used to fund fiscal year 1974 commitments. These additional funds permitted DCRRC to fund 50% of the approved traditional or investigator-initiated research grants; this level of funding was similar to that of the previous year.

The table below shows the total number of applications by type, (i.e., non-competing, competing, etc.) reviewed by the NCI in FY 74. The table indicates the number and dollar amount of applications approved and awarded. The last column indicates the number of unfunded applications.

FISCAL YEAR 1974
(thousands of dollars)

	Requested		Approved		Awarded		Unfunded	
	#	Amount	#	Amount	#	Amount	#	Amount
Type 5 (non-competing renewal)	1398	143,535	1398	143,535	1398	143,535	0	0
Type 2 (competing renewal)	430	72,126	391	39,153	296	33,014	95	6,139
Type 1 (new)	2363	315,289	1622	140,137	1097	90,336	525	49,801
Type 3 (supplemental)	(482)	15,416	(464)	13,169	(456)	11,844	(8)	1,325
TOTAL	4191	546,366	3411	335,994	2791	278,729	620	57,265

Status of NCI Training Programs

At the present time the NIH training programs involve three distinct components, with the potential complications of a fourth in the background.

1. The new postdoctoral NIH Research Manpower Program initially announced by Secretary Weinberger last July.

2. The "old" fellowship and training grant program.

3. Training grants and fellowships which were awarded as a result of the court-ordered release of impounded 1973 funds--representing a sub-set of the old programs.

4. The problem lurking in the background: H.R. 7724.

The new postdoctoral NIH Research Manpower Program was opened to competition in November and received more than 3,000 applications for the initial February 1, 1974, deadline. The NCI received over 600 applications. These are now undergoing competitive review by Initial Review Groups under the aegis of the Division of Research Grants. Awards to the applicants selected will be made before the end of June 1974. A second deadline for receipt of applications for the next round of competition has been set for May 1. Approximately $5.5 million, supporting an estimated 419 awards, will be made available in FY 1974 by the NCI. A similar amount has been budgeted in FY 1975.

The phaseout of the "old" fellowship and training grant programs is continuing. Training grants will decrease from $16.6 million in FY 1974 to $6.1 million in FY 1975. Similarly, old-style fellowships will drop from $635 thousand in FY 1974 to less than $66 thousand in FY 1975.

The court-ordered release of 1973 funds introduced a $4.3 million bulge into the total of FY 1974 training funds. In addition, the Congressional Budget for FY 1974 produced a further influx of funds for training in the second half of the year. In order to reduce high continuation-cost levels in FY 1975, approximately $4.5 million of the 1974 total will be awarded as two-year grants. That is, training grants involved will receive awards from FY 1974 funds which will cover their needs in FY 1975 also.

As of this writing, the status of H.R. 7724 is still in doubt. A conference has been held on the bill, and it is expected that some changes will be made, but their exact nature is not clear. Since the bill, as it went to the conference, substituted a new program for all existing NIH training authorities, its effects on the FY 1976 program will be significant if it is passed.

Released Funds

As a result of the released fiscal year 1973 funds the NCI was able to obligate an additional $7.4 million as follows:

	Amount
Training Program	$4.3 million
Fellowship Awards	$1.5 million
Research Projects	$0.8 million
General Research Support	$0.8 million
TOTAL	$7.4 million

Management and Administration

Several senior staff members have participated in subcommittee meetings of the NIH Program Project and Centers Grants Task Force, initiated by the former NIH Director, Dr. Robert Q. Marston and continued under the current Director, Dr. Robert S. Stone. This Task Force is studying a number of facets of how the various NIH awarding units use the authority to award these types of grants. It is also trying to assess the impact of terminating these large grants and is trying to determine the benefit of the dollars spent in these programs compared to those spent in traditional research programs.

Interagency Agreements

Under the Economy Act of 1932, agencies may provide services for each other when it is clearly advantageous to the Government. A number of our sister agencies possess facilities and expertise profitable for the cancer effort. Currently, we have or are developing agreements with the Atomic Energy Commission, Naval Research Laboratory, and the National Bureau of Standards.

Cancer Control

In a joint effort, the DCRRC and the Cancer Control Program (CCP) administer a grants program in the area of Cancer Control activities. The DCRRC is responsible for review of Cancer Control grant applications through a special review committee being established by the Review and Referral Branch. The CCP is responsible for scientific program management and the DCRRC is responsible for technical merit review and fiscal management.

New Index of Research Grants

The Division issued the first and second editions of the Subject Index of Current Research Grants Administered by the National Cancer Institute, July 1973 and January 1974, respectively. They were produced in a cooperative effort between Dr. Domanski and Mr. Hodge of DCRRC's Biomedical Research Programs Branch and Mr. Holliman of DRG's Documentation Section.

This document, the first of its kind for an individual institute, is divided into three sections. The first is an alphabetical list of research topics with related grant titles and numbers, the second contains a numerical listing of grants, and the third, an alphabetical listing of investigators.

Grant Applications of Secondary Interest to the NCI

Members of the National Cancer Advisory Board requested that NCI staff identify applications of other institutes which may be of interest to NCI. These applications fall into three categories:

1. All dually assigned applications in which the NCI is the secondary assignee and which have been recommended for approval

2. Applications which have not been assigned to NCI but which have been identified by executive secretaries of the various study sections as applications which might be of interest to the NCI because of proposal's indirect relationship to cancer

3. Approved applications which have received exceptionally high priorities (normalized score of about 150 or better) and which have been assigned to institutes other than NCI, but which will go unfunded because of lack of sufficient funds

Accordingly, DCRRC Program Directors have worked closely with DRG Executive Secretaries in identifying and interdigitating appropriate proposals into NCI programs for possible funding.

DCRRC Staff

Dr. J. Palmer Saunders, Director of the Division of Cancer Research Resources and Centers, plans to retire from Government service on June 30, 1974, after 18 years at the National Institutes of Health. He has accepted a position as Dean of the Graduate School of Biomedical Sciences and Professor of Pharmacology in the Medical School at the University of Texas Medical Branch, Galveston, Texas.

Over the past several years it has been the practice of this Division to invite an outstanding individual from the academic radiation therapy community to serve for one year on DCRRC staff. Dr. Frederick George, III, has served in this position since November 1973. He is on sabbatical leave from the University of Southern California School of Medicine where he serves as Director of the Radiation Medicine Section and Professor of Radiology. As a member of the staff of the Clinical Investigations Branch, Dr. George provides leadership, direction and expert guidance to the radiation therapy programs supported by DCRRC, including those found in comprehensive and specialized centers around the country. He also serves in a consultant capacity to other Divisions of the NCI having an interest in radiaiton therapy research.

Serving in Associate Directorships established under the new reorganization of the Division are Dr. Thomas J. King, Acting Associate Director for Research Programs; Dr. John T. Kalberer, Jr., Acting Associate Director for Program Planning; and Dr. John W. Yarbro, Acting Associate Director for Cancer Centers. Also serving in new positions are Dr. Samuel Price, Chief of the National Organ Site Program Branch; Dr. Mordecai Gordon, Chief of the Review and Referral Branch; Mr. Harry Y. Canter, Chief of the Program Analysis and Evaluation Branch; and Dr. Donald Fox, Chief of the Research Facilities and Construction Branch.

New members of the staff include Dr. David L. Joftes, Program Director for the Large Bowel Program; Dr. George M. Willis, Program Director for the Tumor Biology Program; Dr. Barney Lepovetski, Program Director for Review Activities and Executive Secretary for the Cancer Control Grants

Review Committee; and Mr. Kenneth A. Bow, General Engineer in the Research Facilities and Construction Branch. Two staff members who left the Division this year are Dr. Diane Fink, who was Program Director for Chemotherapy, and Dr. George Jay, who served as Chief of the Research Facilities and Construction Branch.

Publication on NCI Grants Programs

During this year, Dr. J. Palmer Saunders, Director of the Division of Cancer Research Resources and Centers, published a paper entitled The Program of the Division of Cancer Grants of the National Cancer Institute. This paper, which is reprinted on the following pages, was published in "Frontiers of Radiation Therapy and Oncology," Vol 8, pp 55-62, 1973.

Front. Radiation Ther. Onc., vol. 8, pp. 55–62
(Karger, Basel and University Park Press, Baltimore 1973)

The Program of the Division of Cancer Grants of the National Cancer Institute

J. P. SAUNDERS

Extramural Activities, National Cancer Institute, Bethesda, Md.

Introduction

In the past decade, the National Cancer Institute has provided support for the development and operation of cancer research centers at educational and research institutions throughout the country. This activity has resulted in a great increase in cancer research, training and education in medical schools as well as in specialized cancer hospitals and research laboratories. Despite this, there still remains a need to create new centers and to strengthen and expand others, and it is for this reason that the President's initiative and the desires of the Congress have emphasized increased authorizations for centers. The general concept of a cancer research center is not something that can be created overnight by edict of the government or of any institution official. It is a concept that can only be realized in full partnership between the efforts of institutions and the medical community.

In 1955, the Congress appropriated sufficient funds for the first large scale attack on the cancer problem which launched the Cancer Chemotherapy National Service Center. That program taught us the procedures and techniques involved in organizing a large-scale government biomedical research effort based on the principle of cooperation and voluntary association of the National Cancer Institute with the scientific community.

On December 23, 1971, the President signed the National Cancer Act of 1971, which authorizes the Director of the National Cancer Institute, with the advice of the National Cancer Advisory Board, to plan, develop and execute an expanded, intensified and coordinated national cancer research program.

Under the act, authority is provided for support of appropriate manpower programs of training in fundamental sciences and clinical disciplines to provide an expanded and continuing manpower base from which to select investigators, physicians and allied health professions personnel, for participation in clinical and basic research and treatment programs relating to cancer including, where appropriate, the use of training stipends, fellowships and career awards.

Comprehensive Cancer Centers

The act provides for the establishment of 15 new centers for clinical research, training, and demonstration of advanced diagnostic and treatment methods relating to cancer.

Cancer center programs identified under section 408A of the National Cancer Act of 1971, hereinafter called comprehensive national cancer research and demonstration centers, ideally will have the following characteristics:

1. The center must have a stated purpose that includes carrying out of clinical research, training and demonstration of advanced diagnostic and treatment methods relating to cancer.

2. The center must have high quality interdisciplinary capability in the performance of diagnosis and treatment of malignant diseases.

3. The center should have or develop an organized cancer detection program.

4. The center must maintain a statistical base for evaluation of the results of its program activities. For this purpose records should be developed which will standardize disease classification to enable exchange of information between institutions.

5. The center should provide leadership in developing community programs involving active participation by members of the medical profession practicing within the area served by the center.

6. The center must have a research base (fundamental and applied) and related training programs with an organizational structure which will provide for the coordination of these activities with other facets of the center program.

7. The center will participate in the national cancer program by integrating its efforts with the activities of other centers in an integrated nationwide system for the prevention, diagnosis and treatment of cancer. For this purpose the center must have sufficient autonomy to facilitate this function.

8. The center must have an administrative structure that will assure maximum efficiency of operation and sound financial practices. The administration should include responsibility for program planning, monitoring and execution as well as preparation of the budget and control of expenditures. Administration and management would include staff appointment and space allocation, the intent being that such a center will have the authority to establish the necessary administrative and management procedures for carrying out its total responsibility as defined in the criteria.

In addition to the comprehensive centers as defined above, the National Cancer Institute also supports specialized centers. Existing examples of such centers may be found in some of the radiation therapy programs which have been established over the past few years. Other examples may be found in center programs which are primarily concerned with chemotherapy of cancer. Existing center programs such as these may be considered part of the comprehensive centers program as defined above, if other qualifying criteria are

met, the emphasis being the participation of such centers in programs of cancer detection, follow-up and evaluation, and the participation of physicians within the referral area in the programs of the center. The specialized centers may at some point form the nucleus about which a comprehensive cancer center may be built or it may continue to exist as a specialized center as a separate and distinct entity or as an integraetd part of a comprehensive center.

Funding

The National Cancer Institute will accept applications for support of all or part of the costs of planning, establishing or strengthening and providing basic operating support for existing or new centers: (1) for clinical research, training and demonstration of advanced diagnostic and treatment methods relating to cancer; (2) construction or renovation, and (3) patient care costs as are required for research.

Support by the National Cancer Institute will be accomplished by the award of grants following formal review as established by the National Cancer Institute and after favorable recommendation by the National Cancer Advisory Board. Support may be provided by one or more grants, the specific proposals in each instance should be submitted after consultation with the staff of the National Cancer Institute.

The initial support for new centers will be for a maximum of 3 years and may be extended for an additional period of 3 years following another formal review. This initial period of up to 6 years will be considered as probationary during which the applicant may demonstrate capabilities.

Since comprehensive clinical centers and program centers provide an established environment for productive work on cancer problems, the efforts of the Division of Cancer Grants activities in the National Cancer Institute will be to enhance the capabilities of existing centers and to establish new mission-oriented programs as a means of increasing benefits from these valuable resources.

The concept of a cancer center is a working together, an integrated effort on the part of all members of the medical community to gain certain objectives and to further the goals of cancer research for the community. It is a concept that requires that the operations of a cancer research center be 'open-ended' and, by its nature, multidisciplinary. The various components, each representing a particular program or programs, should be integrated to the extent that there is a coordinated approach toward solving cancer problems.

As can be seen, centers vary in their stage of development and, at the present time, three types of activities can be recognized. The first is the 'intradepartmental' program in which the attack on cancer is carried out in a single department or entity and is usually concerned either with a fundamental research approach or, if clinical, attuned to a single modality of treatment. Thus, we have radiation therapy programs and programs of chemotherapy in their own particular departments. Such programs of course, while valuable and contributing to cancer research, are limited in their scope and do not concern themselves with the broader aspects of the cancer problem.

The second type is the 'interdepartmental program' in which there is cooperation among several departments, permitting a multiprogrammed approach to cancer. Many of our leading clinical research activities are supported by this type of program and can lead to many sorts of collaborative studies both within and outside of a particular institution. Despite this enlarged scope, this type of program still does not address itself to the full breadth of the cancer problem in a medical community.

The third type of program is the comprehensive, institution-wide program which truly deserves the title of 'Cancer Research Center'. This perhaps is the ideal arrangement and often evolves gradually from the intra- and interdepartmental programs. Its main function is to provide a focus for cancer for the entire medical community. It should serve to provide a close coordination among fundamental scientists, clinical scientists, and practicing clinicians. It should provide such an integrated activity as to permit a rapid two-way communication between laboratory scientists and clinical scientists. In the medical community, it should provide leadership in the prevention, treatment and care of cancer, not by any bureaucratic system of control but by the intelligent practice of close community cooperation in the management of all aspects of cancer. In short, the National Cancer Institute seeks a system which will provide to the maximal extent possible a translation of research results into regular clinical practice, such that no citizen will be denied appropriate professional advice and care because of lack of facilities or knowledge.

Radiation Therapy Clinical Research Centers

This type of center continues to play a very important rôle in cancer management in many major clinical facilities throughout the country. Since it is well recognized that radiation therapy is essential in the management of cancer patients there is continuing interest in developing new radiation centers, particularly in medical schools. This facet of the radiation programs continues to grow, although somewhat slowly. At present 12 of these centers are being funded for a total of over $5,000,000. During this year, six new exploratory projects have been approved that could lead to expansion of existing radiation centers or the creation of new ones. Already a trend is detectable in that at some institutions ongoing radiation research centers are becoming the nucleus for the creation of comprehensive multidisciplinary cancer centers. It appears likely that in the future exploratory projects that originate in radiation therapy departments would be concerned with planning the development of this type of facility as a stepping stone to ultimate development of a comprehensive cancer center. This is a logical evolution since radiation therapists as a group are the physicians most closely identified with cancer in any major medical center.

Training in Radiation Therapy, Radiation Physics and Radiation Biology

Through past efforts of the National Cancer Institute the shortage of trained radiation therapists in the country has lessened. Following a policy of special emphasis of increased training in this field, 36 institutions are receiving support by means of training grants for a total amount just over $3,600,000. Through these grants approximately one-half of all physicians receiving training in radiotherapy at the present time are being supported by the National Cancer Institute. Despite these efforts there continues to be a critical shortage of radiation therapists as shown by a survey conducted by the Committee for Radiation Therapy Studies. It is unfortunate that most ongoing training programs have not sought support from sources other than the National Cancer Institute to continue operation. In view of the limited monies available for training such other sources of support must be sought to allow more new training programs.

A companion manpower problem is the need for training radiation physicists to provide the support required by radiation therapists in carrying out optimal forms of treatment. The American Association of Physicists in Medicine has shown great interest in this problem and grant proposals for training in medical or radiological physics can be anticipated in the future.

Cooperative Clinical Studies

The participation of radiation therapists in cooperative clinical studies continues to increase in a very gratifying fashion. The first such study initiated by the Committee for Radiation Therapy Studies, dealing with preoperative irradiation in lung cancer, has been completed. Other studies dealing with Hodgkin's disease and head and neck cancer are near to completing accession of patients into the studies.

More recently, 25 institutions agreed to form the Radiation Therapy Oncology Group (RTOG) to carry out cooperative clinical trials. This group has been reviewed favorably by the Cancer Clinical Investigation Review Committee and the National Cancer Advisory Board and is now receiving funds for its activities. At the present time this group is involved in projects studying irradiation of brain metastasis, split course of irradiation, and the effectiveness of hyperbaric oxygen and oxygen breathing in conjunction with radiation therapy for a variety of malignancies.

The creation of the RTOG is very encouraging since it will likely lead to a more meaningful dialog with other groups already participating in cooperative clinical trials under the aegis of the Clinical Investigations Branch. It is expected that the RTOG will come forth with additional protocols and, also, will help some other cooperative clinical groups in the preparation of protocols involving radiation therapy.

Research Grants

This category covers generally unsolicited research grants. At present about 60 grants of this type are being supported through this program. The total amount is just over $3,000,000. The scientific content of this category is broad spectrum, from the very basic to the quite applied. It cuts across the disciplines of physics, chemistry and biology. Basic studies include investigations into the molecular basis for radiation lethality and biochemical changes caused by radiation inactivation. Applied studies include computer analyses of tumor roentgenograms and optimizing automated radiation treatment planning. Physics studies include proton energy loss measurements. Chemistry studies include photochemistry of biologically important bases. Biology studies include RNA synthesis in mammalian cells after irradiation. The overall goal of this funding mechanism in the radiation program is to broaden the understanding of the interaction of radiation with biological material and to increase the impact of such information on the cure and prevention of cancer.

High Energy Studies

Another important goal in the radiation programs over the next few years is the assessment of the role of high linear energy transfer (LET) particle radiation in cancer therapy. There is already considerable experimental data suggesting that low oxygen tension renders mammalian cells less radiosensitive to the effects of γ-ray or û-radiation. Since most human tumors contain necrotic foci, it is very likely that at least part of the tumor population is hypoxic, which could account for many radiation failures. In fact it has been estimated that annually there are approximately 50,000 patients treated by radiation in whom the initial manifestation of recurring disease is at the site of the primary tumor. There is a growing consensus that many, if not most, of these local failures are due to the hypoxic tumor population which, if overcome, could yield dramatic improvements in cure rates. Excitement over high LET radiation is based on growing evidence that it may overcome the radioresistance of hypoxic tumor cells. A pilot study being carried out at Hammersmith Hospital in London appears to substantiate this for fast neutrons. In order to confirm these observations, if they are real, the National Cancer Institute is supporting several research projects in this country, using cyclotron-produced fast neutrons, that should lead to clinical trials in the near future. In addition, support has been provided for projects that studied the feasibility of designing a deuteron-tritium generator for the production of neutrons that could provide a clinically suitable source of neutrons for cancer therapy.

Another form of high LET particle radiation which seems to offer similar biologic advantage is negative Π-mesons. In collaboration with the Atomic Energy Commission the National Cancer Institute is funding the design and construction of a biomedical channel at the Los Alamos Meson Facility to study the possible clinical applications of this type of particle.

During fiscal year 1972 approximately $1,000,000 is being spent on high LET radiation efforts and it is anticipated that during fiscal year 1973 over $2,000,000 will be spent in this area.

OFFICE OF THE ASSOCIATE DIRECTOR
PROGRAM PLANNING

The Office of Program Planning was established in April of 1973 to meet the growing needs for planning and evaluation of grant-supported programs administered by the Division of Cancer Research Resources and Centers (DCRRC). It is headed by the Acting Associate Director for Program Planning and includes two branches--the Program Analysis and Evaluation Branch and the Program Development and Operations Branch. The following report describes the activities of the immediate office of the Acting Associate Director, which is also assuming the responsibilities of the Program Development and Operations Branch until additional staff can be hired. The report of the Program Analysis and Evaluation Branch follows later.

The Office of Program Planning (OPP) integrates data from various sources within the Division in order to establish a broad overview of past trends and present and future needs. It also serves the National Cancer Institute (NCI) as a whole by maintaining a close working relationship with the Office of Program Planning and Analysis (OPPA) in the Office of the Director, NCI.

To help provide the professional manpower needed to fulfill these roles in the Division and the Institute, the OPP administers and manages several contracts with outside firms and cooperates with the OPPA in several other contract-supported studies. A summary of tasks accomplished with the aid of these contracts follows.

Program Planning and Services for DCRRC

Capital Systems Group, Inc. (CSG), located in Bethesda, Maryland, assists the Division on a day-to-day basis in planning and evaluation of programs; collection, analysis and presentation of data; preparation of publications; and planning and management of conferences. The Acting Associate Director for Program Planning is Project Officer and the services afforded by the contractor are available to all personnel in DCRRC.

Since the beginning of the contract in December 1973, CSG has played a significant role in a variety of projects related to DCRRC programs. Included in these projects are evaluation of computer methodology for possible use in the Program Analysis and Evaluation Branch; determination of projected needs of many of the DCRRC programs; analysis of DCRRC program data; art work and visual aids for several formal presentations; several publications describing different programs of the Division; and support of conferences for the Biomedical Research Programs Branch; Clinical Investigations Branch, and Cancer Centers Branch.

Planning for Cancer Centers

With the aid of two contracts, the OPP, in conjunction with the Cancer Centers Branch, is studying some of the factors necessary for an effective and

efficient Cancer Centers Program. Since November 1973, JRB Associates in La Jolla, California, has worked on a project to determine the optimal location, on the basis of population, of cancer centers in the United States.

It was first determined that national Congressional districts were an appropriate unit in which to uniformly divide the population of the United States. Using a computerized mathmatical model, the contractor, in consultation with the Acting Associate Director, OPP, defined the optimal locations of cancer centers on the basis of the distance the population of a Congressional district would have to travel to reach the nearest comprehensive center. These optimal locations, in turn, depend on the number of comprehensive cancer centers open. Data in a preliminary report indicate the various optimal locations on the basis of between 14 and 37 centers. The authors of the report are now preparing to complete this study and to extend it to develop a similar model system based on the location of medical schools throughout the country.

CDP Associates, Incorporated, also located in La Jolla, California, has developed a model system for evaluation of the impact of comprehensive cancer center programs on the regions they serve. In a preliminary report, the contractor presented the factors considered in establishing the methodology for the impact evaluation program, the various tasks involved in carrying out such a program, and an estimation of the manpower and funds needed for each task.

The proposed system provides for long-range information collection as a by-product of each program operation in the centers and employs a statistically designed "random" sampling information system. Such information will be used for evaluating the effectiveness of the cancer centers and for determining changes that must be made to improve their role in treatment of cancer patients and education of the medical community and the public in matters related to cancer.

Planning and Manpower Utilization

The OPP has been cooperating with the Office of Administrative Management, NCI, to determine as precisely as possible the number of positions needed to efficiently manage the DCRRC grants programs. This manpower utilization study is being done in response to a directive from the Office of the Secretary, Department of Health, Education, and Welfare (DHEW), requesting NCI to evaluate and forecast manpower requirements for its grants and contracts programs. The NCI study, which utilizes a modification of the "Manpower Measurement, Forecasting and Control System" developed by DHEW, will soon be completed.

Management Information System

The Office of Program Planning, DCRRC, worked closely with the Office of Program Planning and Analysis, NCI, to help develop a Management Information System (MIS) for the Institute. TRW Systems Group, McLean, Virginia, is the contractor assisting the NCI Planning Office in this effort.

As one of the initial projects, TRW determined the information available from DCRRC to feed into the MIS. Through the cooperation of the OPP and other members of DCRRC staff, the contractor learned about the grants process, organization and function of DCRRC, and information activities already established in the Division.

The MIS, still in the preliminary stages of development, is designed to coordinate all administrative and financial data from the four NCI Divisions and the Office of the Director. It is anticipated that the MIS will provide NCI management personnel with a quick source of information needed to make timely decisions on redirection of program thrusts and funding and reallocation of personnel and other resources required to accelerate programs of cancer research and cancer control.

Planning Workshop for Cancer of the Pancreas

The publication on the following pages describes the proceedings of the DCRRC/NCI-sponsored planning workshop for study of cancer of the pancreas, held in March 1973. It is reprinted from the Journal of Surgical Oncology, Vol. 6, No. 1, January 1974, pp. 1-8.

Cancer of the Pancreas

JOHN T. KALBERER, JR., Ph.D.

The National Cancer Institute sponsored a one-day workshop to discuss future plans for the study of pancreatic cancer. Ten participants discussed the limitations in progress against cancer of the pancreas and recommended studies in the areas of fundamental research, diagnosis, and treatment. They emphasized the need for coordination of clinical studies across the country, development of an early detection method, and participation of more biochemists to better define pancreatic pathophysiology.

The age-adjusted mortality rate from pancreatic cancer has more than tripled in the United States during the last 50 years (Statistical Research Service, 1970). Treatment for cancer of the pancreas has been ineffective as indicated by a mean survival from time of diagnosis of less than 5 months (Moldow, 1968). These facts have prompted the National Cancer Institute (NCI), one of the institutes of the National Institutes of Health to actively pursue new methods of diagnosis and treatment of pancreatic cancer and to encourage additional fundamental research in this area. As one step in this direction, the NCI sponsored a one-day informal working group meeting (Table I) on March 6, 1973, in Bethesda, Maryland, to discuss future plans for the study of this dease

PURPOSE OF MEETING

The meeting was opened by the Chairman, Dr. Edward Holyoke, and events leading up to this meeting were reviewed. These included increased public interest and requests from several members of the National Cancer Advisory Board.

Dr. Kalberer explained that the NCI decided to hold a workshop meeting so that participants might formulate new ideas on what direction the NCI and the scientific community might take to further the studies of cancer of the pancreas. The meeting was purposely unstructured and the consensus of opinion, as expressed by one participant, was that "the free-wheeling atmosphere allowed people to voice their opinions, doubts, questions, without the need for precise wording (which often serves to confuse) inherent in more formal presentations." A meeting that included formal presentations of papers for exchange of information

Division of Cancer Grants, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

Journal of Surgical Oncology
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TABLE I. Participants at the National Cancer Institute's Pancreas Working Group Meeting, March 6, 1973

Dr. Stephen K. Carter Chief, Cancer Therapy Evaluation Branch Division of Cancer Treatment, NCI	Dr. John T. Kalberer, Jr. Associate Director for Planning Division of Cancer Grants, NCI
Dr. Isadore Cohen Prof. and Ch., Dept of Surgery State University School of Medicine New Orleans, Louisiana	Dr. Thomas King Program Director, Bladder-Prostate Program Division of Cancer Grants, NCI
Dr. Diane Fink Program Director, Chemotherapy Division of Cancer Grants, NCI	Dr. Samuel Price Program Director, Large Bowel Program Division of Cancer Grants, NCI
Dr. Edward D. Holyoke Chief, Dept. of General Surgery Roswell Park Memorial Institute Buffalo, New York	Dr. Frank E. Rosato Prof. of Surgery Hospital of the University of Pennsylvania Philadelphia, Pennsylvania
Dr. Robert Johnson Director, Division of Clinical Oncology University of Wisconsin Madison, Wisconsin	Dr. Avery Sandberg Chief of Medicine C Dept. of Medicine Roswell Park Memorial Institute Buffalo, New York

had been considered, but since a meeting of this type was held only last May (1972) in Clearwater, Florida, there would have been little additional information.

The participants stressed that improvement in the presently dismal results being obtained with pancreatic cancer depends on increased research efforts, both clinical and fundamental. However, current NCI research support in this area is meager by virtue of the fact that there are few if any leads. Consequently, both clinical and basic scientists are reluctant to pursue this area of research.

This dilemma is reflected in the dollars awarded by the NCI in support of research on pancreatic cancer in fiscal year 1973 – a total of less than 2 million dollars (Table II). This figure drops to less than 1 million dollars if we consider only those grants and contracts used to support research on pancreatic cancer alone; ¾ of this money is being used for the newly initiated contract for combined modality study of adenocarcinoma of the pancreas.

When we look at the NCI Clinical Cooperative Studies Program, we find that of 128 cooperative protocols, only 3 are committed exclusively to trials on cancer of the pancreas. If we examine the phase II studies, we find that 41 of the multiple solid tumor protocols are involved only incidentally with cancer of the pancreas.

Even more critical is the fact that only one research project grant deals exclusively with basic research on cancer of the pancreas. Consequently, the NCI is making every effort to encourage additional scientists to work in this area, especially biochemists, pathologists, and immunologists.

When the review on research support was completed there followed a brief review on what is known about this disease.

TABLE II. National Cancer Institute Support for the Study of Cancer of the Pancreas

Type of Support	Percent of Emphasis on Cancer of the Pancreas	Number of Grants or Contracts	Estimated Amount in Dollars
Traditional project grant	100%	1	$ 29,347
Traditional project grant	25% or less of total effort	20	450,000
Cancer research center grant	5% or less of total effort	9	175,000
Clinical cooperative studies, contract	100%	5 institutions participating in the Gastrointestinal Tumor Study Group	720,000

	Group	Pancreas directed protocol(s)	Total group protocols	Multiple* solid tumors (incidentally may include pancreas)	Total group protocols	Estimated Amount in Dollars	
Clinical cooperative studies grant	Central Oncology Group	1/17		9/17		$ 80,000	
	Eastern Cooperative Oncology Group	2/28		2/28		130,000	
	Southern Group	0/15		5/15		35,000	
	Southwest Cancer Chemotherapy Group (Adults)	0/40		15/40		90,000	
	Western Cancer Study Group	0/28		10/28		45,000	380,000
	Total	3/128		41/128		$380,000	

*Phase II trials (treatment of specific types of cancer, chiefly organ related).

GRAND TOTAL $1,754,347

CANCER OF THE PANCREAS: A BRIEF SUMMARY

Morbidity and Mortality

The death rate from cancer of the pancreas has been increasing steadily in the United States since 1920 (Statistical Research Service, 1970). Pancreatic cancer accounts for about 15% of all cancers of the digestive system and is the fourth most common cause of cancer deaths in the United States. Latest estimates indicate that 19,400 Americans develop this disease each year – 11,000 men and 8,400 women – and that 19,200 persons die from it annually (Cancer Facts and Figures, 1973).

Cancers of this type rarely occur in persons under 25 years of age and are most common between the ages of 30 and 70 (Ackerman and del Regato, 1970). The incidence increases steadily with age and is higher in the black population than the white population (Segi and Kurihara, 1964).

Etiology

Causes of pancreatic cancer have not yet been determined, but epidemiological studies have provided some clues to causes and related factors. An association between diabetes and pancreatic cancer has been reported but diabetes seems to increase the predisposition to all cancers (Bell, 1957). Data on the association of chronic pancreatitis and pancreatic cancer are conflicting. However, neither chronic pancreatitis nor diabetes has increased at the same rate as cancer of the pancreas (Krain, 1970).

Heavy cigarette smoking, chronic alcoholism, and exposure to certain industrial carcinogens such as coal tar, beta-naphthalamine, and benzidine, have also been associated with increased incidence of this disease (Krain, 1970).

Cancers of the pancreas vary widely in histologic type. However, few epidemiological studies distinguish between these types. The term "adenocarcinoma" can apply to tumors derived from any one of at least 5 cell types which comprise over 95% of the pancreas, but in practice it usually refers to one of the more common ductal cell types.

The following classification of exocrine pancreatic carcinomas (in approximate order of frequency) has been suggested by Frantz (1959), who also discusses several unusual variants not listed in this scheme.

I. Adenocarcinoma
- Small duct (often scirrhous)
  - Variants of small duct carcinoma:
    - Poorly differentiated
    - Colloid carcinoma (mucoid)
- Large duct
  - Variants of large duct carcinoma
    - Papillary adenocarcinoma
    - Muco-epidermoid carcinoma
    - Squamous cell carcinoma
    - Cystadenocarcinoma
- Acinar cell (medullary)

II. Pleomorphic (indeterminate)
- Spindle cell form
- Giant cell form
- Carcinosarcoma

Diagnosis

Symptoms of pancreatic cancer, predominately pain, jaundice, and weight loss, depend on whether the tumor is located in the head, body, or tail of the organ. They are not unique for this disease but may mimic signs of other gastrointestinal tract disorders such as peptic ulcer, cholelithiasis, or pancreatitis, and usually they do not become severe enough to demand attention until the cancer is well advanced.

At present, diagnosis of cancer of the pancreas at an early, curable stage is extremely difficult and depends on a high degree of suspicion. Tests specific for the detection of pancreatic cancer have not yet been developed. However, suspicions of pancreatic cancer may be supported by nonspecific tests such as pancreatic function tests (analysis of enzyme secretions, bicarbonate level, etc.), upper GI series, arteriography, endoscopic techniques, radioisotope scanning, ultrasonic scanning, and operative radiological examination. Surgery is often useful in confirming a tentative diagnosis.

Therapy

Most investigators express the view that the chance for curing pancreatic cancer are exceedingly slim. However, if a pancreatic tumor is found to be localized and if the adjacent tissues contain only limited or no metastases, surgical removal of the tumor seems to offer the nearest approach to a "cure."

The surgical procedures used for possible cure are total pancreatectomy and pancreatiduodenectomy (Whipple procedure). These operations are quite radical and postoperative mortality rates are high.

For the Whipple procedure, for example, the mortality rate varies between 12 and 50%. This procedure is a formidable one and the best results depend on extensive preoperative and postoperative care along with a surgeon of outstanding ability and experience.

Only about 8% of patients with pancreatic cancer are eligible for these radical procedures. When the tumor is not resectable and metastatic involvement is very extensive, then palliative chemotherapy and radiation are frequently used as adjuvants to palliative surgical techniques, such as bypass procedures. Patients receiving palliative treatment live about 2.5 to 8 months after surgery.

Except for the treatment of islet cell carcinoma with streptozotocin, chemotherapy has not produced any significant results to date. The NCI, in an effort to develop new methods of treatment, has established a Gastrointestinal Tumor Study Group. This group consists of 5 separate institutions engaged in common protocol studies to be applied to adenocarcinoma of the pancreas.

Two protocols have been planned and developed by this group. One is a prospective, randomized, phase III study using 5-fluorouracil (5-FU) and radiation therapy as an adjuvant in patients with potentially curable pancreatic cancer. The other protocol involves a controlled evaluation of high-dose (6000 rad) radiation therapy, high-dose radiation plus 5-FU, and medium-dose (4000 rad) radiation therapy plus 5-FU in patients with localized but unresectable disease (Carter, 1973).

Radiotherapy has not been encouraging and at best is only palliative. The palliative effect occurs mostly through relief of pain and decrease of jaundice (Ackerman and del Regato, 1970).

RECOMMENDATIONS

Considering these limitations in the state-of-the-art of pancreatic cancer, the members of the working group made specific recommendations for future study. They also emphasized the importance of coordinated clinical research efforts across the country and the need to bridge the communications gap between fundamental scientists and clinicians.

Basic Research

The participants, all clinicians, strongly urged the participation of more biochemists in the study of pancreatic cancer. They believe that biochemists can make a valuable contribution to our understanding of pancreatic function, which will result in the development of more effective methods of diagnosis and treatment. Some of the recommended areas of study included:

1. Mechanism of action of hormones on pancreatic cells (may lead to diagnostic tools such as radioactive polypeptides for scintigrams and treatment methods such as chemotherapeutic agents attached to hormones that concentrate in the pancreas)
2. Role of pancreatic enzymes
3. Development of a model system
4. Immunology
5. Carcinogenesis

Diagnosis

Since at least 80% of pancreatic cancers are diagnosed too late for effective treatment, the participants emphasized the importance of developing methods for early diagnosis of pancreatic cancer and determining high risk population groups. They recommended further study in the following areas:

1. Fiberoptic endoscopy
2. Specific biochemical markers such as carcinoembryonic antigen, enzymes, fibrinogens
3. Angiography
4. Scanning techniques
5. Exploratory research

Treatment

Members of the working group stated that development of surgical techniques has reached its limit for treatment of pancreatic cancer. Since these techniques are so complicated, the next important step is to make sure that surgeons treating patients for cancer of the pancreas be very experienced. This requirement may be met by the establishment of a few pancreatic surgical centers. Such a measure may well improve postoperative mortality rates.

Development of new drugs and continued study of clinical chemotherapy,

both alone and combined with other treatment methods, was greatly encouraged. Dr. Stephen Carter described studies of cancer of the pancreas that are being funded by the NCI. Some of these studies are described above and the full text of Dr. Carter's presentation appears in this issue.

The study of immunotherapy in combination with other forms of treatment was recommended as well as the study of the immunocompetence of patients with pancreatic cancer to try to determine why such patients deteriorate so rapidly and to measure their response to therapy.

FUTURE MEETING

A second meeting, to which biochemists and radiation therapists will be invited, will be held in 1974. An unstructured format will be used again to encourage exchange of ideas and opinions rather than simply exchange information.

ACKNOWLEDGMENTS

The author wishes to extend his deep appreciation to Mrs. Toby Pick for her significant contributions in the preparation of this paper. Also, he wishes to thank Mr. Harry Canter and his Program Analysis and Reporting Section for help in collecting data and Mrs. Bernice Leslie for typing the manuscript.

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Presentation to the National Cancer Advisory Board

Dr. John T. Kalberer, Jr., Acting Associate Director for Program Planning, presented the Status Report on Grant-Supported Research Programs to the National Cancer Advisory Board on March 19, 1974. Included in his presentation were the following charts and tables, which reflect funding trends over the past ten or more years of grant-supported activities administered by the DCRRC/NCI.

Of special interest is the effect of the National Cancer Act of 1971 on these trends. Where appropriate, the charts and tables include comparisons between NCI funding data and those of other institutes of NIH. A summary of the conclusions drawn from the data follows the charts.

LIST OF
CHARTS AND TABLES

NCI Programs Fiscal Year 1973, Actual Obligations $430,427,000

DCRRC Programs Fiscal Year 1973, Actual Obligations $213,493,000

Traditional Grants, Center Grants, and Exploratory Projects, Number of Grants and Dollars Awarded

NCI Obligations

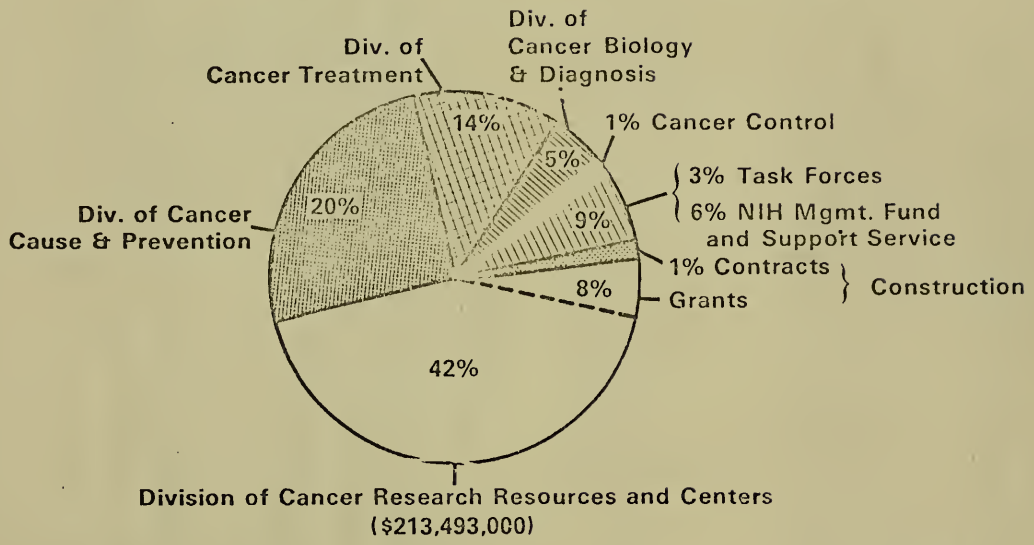
FY 1973 Actual Obligations, Distribution of Funds

Age of Applicants and Funding Status for Traditional Grants (New), FY 73

Institutions Receiving More Than $500,000 in NCI Research Grant Funds, FY 73

Summary

NCI PROGRAMS Fiscal Year 1973
Actual Obligations $430,427,000



DCRRC/NCI
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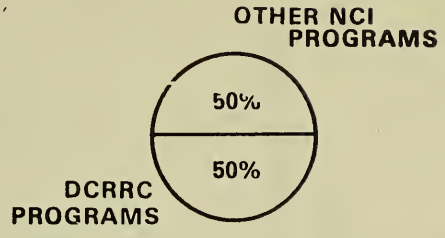
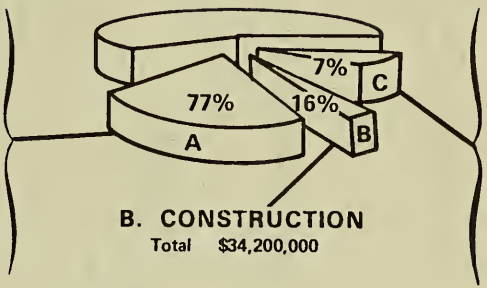
DCRRC PROGRAMS Fiscal Year 1973
Actual Obligations $213,493,000



A. RESEARCH GRANT PROGRAMS

Traditional	43%
Centers	40
Clinical Trials	8
Gen'l Research Support	4
Task Forces	2
Supportive Care	2
Exploratory	2
Scientific Evaluation	0.3
Total	$163,587,000



B. CONSTRUCTION
Total $34,200,000

C. TRAINING GRANT PROGRAMS

Graduate	48%
Clinical	34
(medical 31%)	
(dental 3%)	
Fellowships	18
Total	$15,706,000

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NCI PROGRAMS, FISCAL YEAR 1973
Actual Obligations, $430,427,000

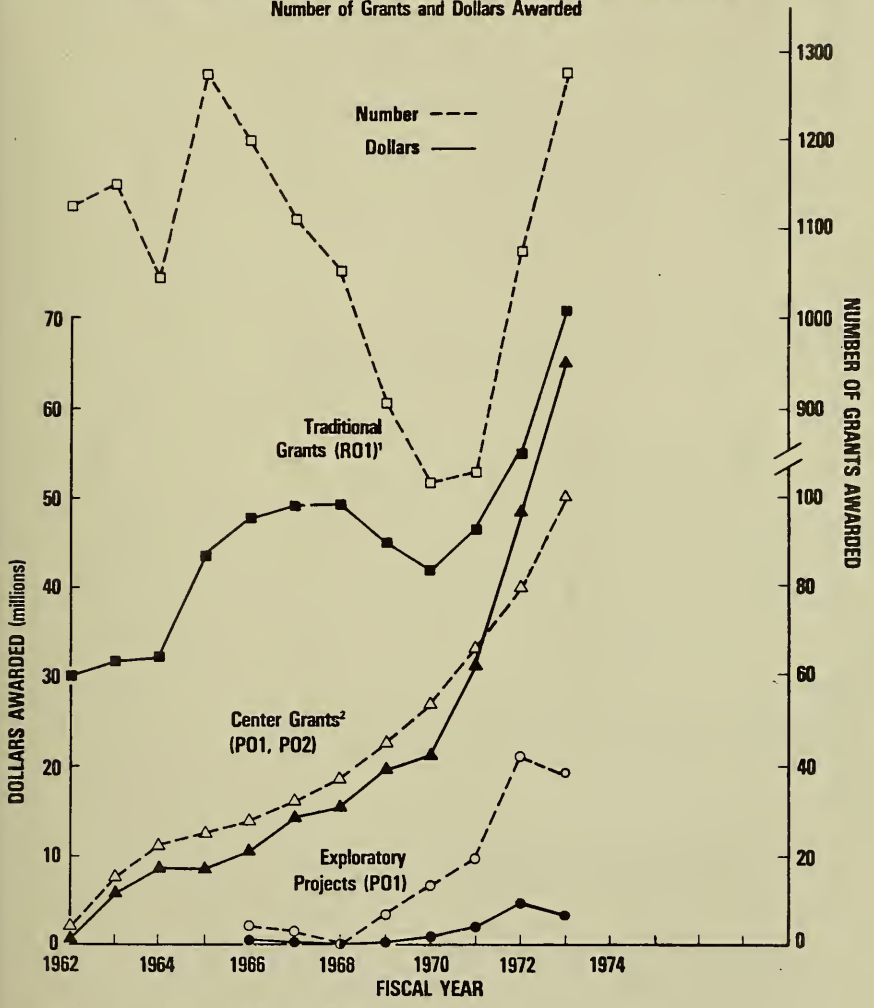
NCI Actual Obligations	$ (Thousands)	Actual %	Rounded Off %
Div. of Cancer Research Resources & Centers (includes construction grants)	213,493	49.6	50
Div. of Cancer Cause & Prevention	87,688	20.4	20
Div. of Cancer Treatment	58,803	13.7	14
Div. of Cancer Biology & Diagnosis	22,827	5.3	5
Div. of Cancer Control	5,222	1.2	1
NIH Management Fund & Support Svcs.	26,615	6.2)	6)
Task Forces	11,175	2.6) 8.8	3) 9
Construction Contracts	4,067	0.9	1
Molecular Control	537	0.1	0
	$ 430,427,000		

Division of Cancer Research Resources and Centers - Actual Obligations			
Research Grant Programs	$ 163,587,000	76.6	77
Traditional	69,585	42 5	43
Centers:	65,010	39.7	40
Core $13,282 (20%)			
Operational Support 51,728 (80%)			
Clinical Trials	12,791	7.8	8
General Research Support	5,924	3.6	4
Task Forces	3,950	2.4	2
Supportive Care	3,292	2.0	2
Exploratory	2,500	1.5	2
Scientific Evaluation	535	0.3	0.3
Construction	$ 34,200,000	16.0	16
Training Grant Programs	$ 15,706,000	7.4	7
Graduate Research	7,576	48.2	48
Clinical	5,324	33.9	31 Medical
			3 Dental
Fellowships	2,806	17.9	18
	$ 213,493,000		

NCI

TRADITIONAL GRANTS, CENTER GRANTS, and EXPLORATORY PROJECTS

Number of Grants and Dollars Awarded



[1]Traditional Grants include SKI for FY66 through 71

[2]Center Grants include SKI for FY72 and 73

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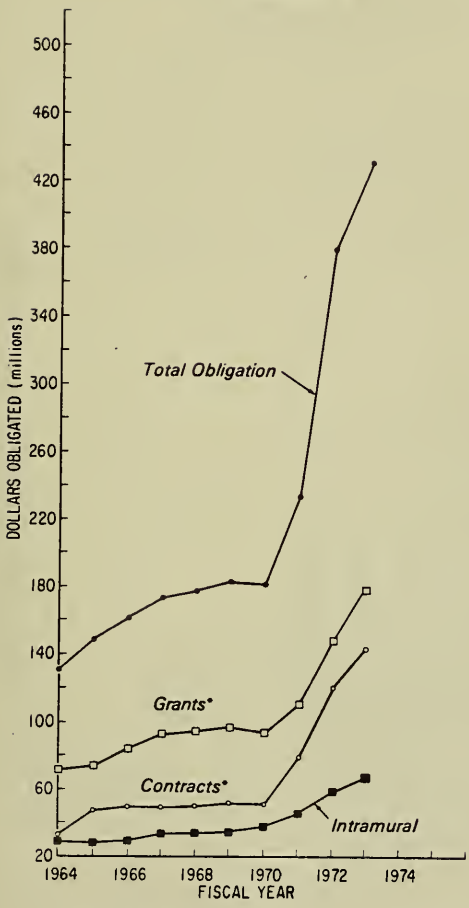
NCI TRADITIONAL GRANTS, CENTER GRANTS, AND EXPLORATORY GRANTS
Number of Grants and Dollars Awarded (Millions)

Fiscal Year	TRADITIONAL GRANTS			CENTER GRANTS			EXPLORATORY GRANTS	
	No.	Amount Excluding SKI $	Amount Including SKI $	No.	Amount Excluding SKI $	Amount Including SKI $	No.	Amount $
1962	1127	30.2		4	0.6			
63	1150	31.9		15	6.0			
64	1058	32.1		22	8.3			
65	1279	43.5		25	8.3			
66	1200	43.9	47.8	28	10.4		4	0.3
67	1112	45.2	49.3	32	14.4		3	0.1
68	1057	45.1	49.4	37	15.5		0	0
69	907	40.7	45.0	45	19.7		7	0.2
70	818	37.4	41.9	53	21.1		13	0.8
71	828	41.6	46.6	66	31.3		19	1.9
72	1072	55.0		81	42.1	48.6	42	4.6
73	1285	63.5		100	56.4	65.0	39	3.7

NCI OBLIGATIONS



*Excludes Construction

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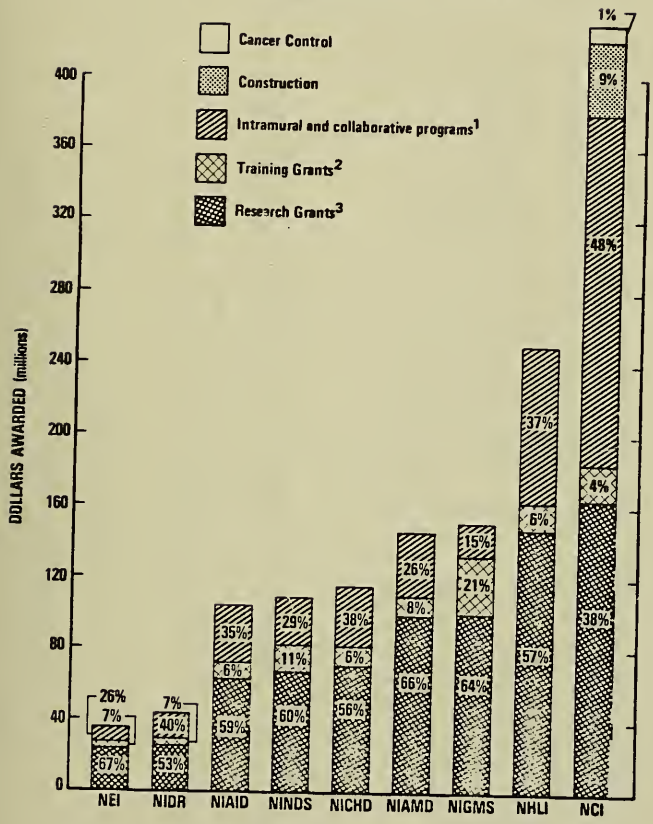
NCI OBLIGATIONS
(Thousands)

Fiscal Year	TOTAL	OBLIGATIONS					
		GRANTS		CONTRACTS		INTRAMURAL	CONSTRUCTION
		Excluding Construction	Including Construction	Excluding Construction	Including Construction		Total Additional
1964	$131,325	$ 70,691	$	$ 32,114	$	$28,520	$
1965	147,995	72,752		47,533		27,710	
1966	161,313	82,798		49,467		29,048	
1967	173,342	92,067		48,534		32,741	
1968	177,346	94,231		49,543		33,572	
1969	182,436	96,054		51,819		34,563	
1970	181,352	92,846		50,833		37,673	
1971	233,073	109,887		78,197		44,989	
1972	378,636	146,623	(193,627)	122,049	(126,048)	58,961	(51,003)
1973	430,427	179,293	(213,493)	141,130	(145,197)	66,515	(38,267)

FY 1973 ACTUAL OBLIGATIONS
Distribution of Funds



[1] Includes Intramurel Research, Research & Research Support, and NCI Management and NIH Management Fund

[2] Includes Graduate Training Grants, Clinical Training Grants, Fellowships, RCA, and RCDA

[3] Includes Regular Research Grants, Specialized Research Centers, Leukemia Treatment Centers; Excludes General Research Support, Drugs, Scientific Evaluation

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FY 1973 ACTUAL OBLIGATIONS
DISTRIBUTION OF FUNDS

INSTITUTE	FY73 ACTUAL OBLIGATIONS $ (000's)	RESEARCH GRANTS [1] %	TRAINING GRANTS [2] %	INTRAMURAL AND COLLABORATIVE [3] %	CONSTRUCTION %	CANCER CONTROL %
NEI	34,389	67	7	26		
NIDR	40,864	53	7	40		
NIAID	103,025	59	6	35		
NINDS	107,368	60	11	29		
NICHD	111,207	56	6	38		
NIAMD	142,814	66	8	26		
NIGMS	154,035	64	21	15		
NHLI	255,722	57	6	37		
NCI	430,427	38	4	48	9	1

[1] Includes Regular Research Grants, Specialized Research Centers, Leukemia Treatment Centers; Excludes General Research Support, Drugs, Scientific Evaluation

[2] Includes Graduate Training Grants, Clinical Training Grants, Fellowships, RCA, and RCDA

[3] Includes Intramural Research, Research and Research Support, and NCI Management and NIH Management Fund

AGE OF APPLICANTS AND FUNDING STATUS FOR TRADITIONAL GRANTS (NEW) NCI — FY'72

FUNDING STATUS

PRINCIPAL INVESTIGATOR 35 YEARS OF AGE AND UNDER

	#	%	$(MILLIONS)	%
APPROVED FUNDED	84	52	3.1	56
APPROVED UNFUNDED	21	13	0.4	7
DISAPPROVED	57	35	2.0	37
TOTAL	162	100	5.5	100

AVERAGE APPROVED FUNDED AWARD = $36.4 THOUSAND

PRINCIPAL INVESTIGATOR OVER AGE 35

	#	%	$(MILLIONS)	%
APPROVED FUNDED	194	32	9.2	35
APPROVED UNFUNDED	80	13	2.3	9
DISAPPROVED	325	55	15.1	56
TOTAL	599	100	26.6	100

AVERAGE APPROVED FUNDED AWARD = $47.8 THOUSAND

PROGRAM ANALYSIS AND EVALUATION BRANCH
DCRRC/JUNE 1973

INSTITUTIONS RECEIVING MORE THAN $500,000 IN NCI RESEARCH GRANT FUNDS FY 1973

	INSTITUTIONS		GRANTS		DOLLARS	
	NO.	%	NO.	%	$	%
TOTAL	287	100	1885	100	$151,771,000	100
>$500,000	74	26	1337	71	$130,741,000	83

DCRRC/NCI
2/74

INSTITUTIONS RECEIVING MORE THAN $500,000 IN NCI RESEARCH GRANT FUNDS (excluding GRSG, DRUG, and CONSTRUCTION) - FY 73

GROUP	$ (000's)	%	No. of Grants	%
1-10	53,013	33.6	414	22.0
11-20	24,511	15.5	199	10.5
21-30	19,151	12.1	246	13.0
31-40	11,450	7.3	136	7.2
41-50	7,949	5.0	88	4.7
51-60	6,869	4.4	124	6.6
61-70	5,766	3.7	96	5.1
71-74	2,032	1.3	34	1.8
TOTAL	$130,741	82.9	1,337	70.9

Summary:

1. 287 institutions received research funds (excludes training and fellowships)

2. 74 institutions constitute 25.8 percent (74 ÷ 287) that received over $500,000 in research grant funds.

3. 1,337 grants received by these same 74 institutions constitute 70.9 percent (1337 ÷ 1885) of all such grants.

4. $130,741 received by these same 74 institutions constitute 82.9 percent ($130,741 ÷ $157,771) of all such funds.

SUMMARY

1. Grants program was responsible for 50% of total FY 73 budget.

2. There are three major areas of DCRRC funding:

    a. Research Grants - 77%
    b. Construction - 16%
    c. Training - 7%

3. The three major areas of research grants funding are:

    a. Traditional grants - - - - - - - - - - - - - - - - - 43%
    b. Centers Program including Program Project grants - 40%
    c. Clinical Cooperative Trials- - - - - - - - - - - - 8%

4. Centers program has increased at a greater rate than the traditional grants program.

5. With passage of National Cancer Act of 1971, the budget for grants has increased 93%.

6. The Act has stimulated a three-fold increase in new applications reviewed.

7. Approval rate for traditional grants is greater for renewal than new.

8. The award rate for grants has improved with passage of the Act, with traditional grants receiving 50% funding.

9. Young investigators have competed well for NCI grant funds.

10. Of institutions receiving NCI support, 26% receive 71% of the total grants and 83% of the total dollars awarded.

PROGRAM ANALYSIS AND EVALUATION BRANCH

This year there has been continued emphasis placed on the dissemination of information related to the science of the several granting programs. One of the major functions of the Branch is to assist others, including the staff of all the NCI Divisions, in keeping abreast of the research which is supported by the Division of Cancer Research Resources and Centers (DCRRC). This is an essential function if the NCI is to be effective in coordinating and managing the National Cancer Plan. The Branch conducts six major programs designed to inform others of the types of research projects being funded. Each of the NCI Divisions receives, on a continuing basis throughout the year, a summary of each new grant that is awarded as well as a summary of each successfully competing continuation grant. As reprints from grantees are received in the Branch, copies are distributed to other NCI Divisions. One of the major operations of the Branch is the publication of the NCI Grant Supported Literature Index. A copy of the Index is sent to every Branch and Laboratory Chief and each Section Head within the NCI. The Index will be discussed in greater detail later. Special narrative reports are prepared during the year on topics of current interest and are given wide distribution within the NCI. During the past year the following reports were prepared:

Chalones: Present Concepts and Possibilities for Clinical Cancer Therapy

Rehabilitation In The National Cancer Program Plan

Trends In Cancer Epidemiology

The Application of High-Linear-Energy-Transfer Radiation for Improved Cancer Therapy.

Immunotherapy - 1973

Inhibition of Chemical Carcinogenesis

Special reports are prepared at the request of persons interested in particular aspsects of the grant programs. The most recent request dealt with active NCI grants supporting investigations in the field of cancer immunology. The grants in this area were selected and grouped into fourteen subdivisions for easier handling and better analysis. One of the major tasks of the Branch each year is the preparation of the Appendix to the Division's Annual Report (see Appendix attached to Annual Report). This is a comprehensive narrative review of the literature published by NCI grantees during the past year. The preparation of this report requires year-round surveillance of the literature and ability to relate and organize basic and clinical data.

Another important function and one which requires an ever increasing amount of time and effort is the review of pending grants and contracts, to assure that there is no overlap or duplication between these two forms of NCI support. The increase in funding of both grants and contracts has been felt in this

operation. The Branch is currently studying the applciation of a computer-based data system (see section on MIS) and it is hoped that this system will help alleviate some of this increased burden.

Journal scanning of approximately 250 selected journals is performed by the Branch to produce a monthly compilation of papers published by NCI grantees, fellows, and trainees. Information collected is used both in report writing and answering questions submitted by the Division. The most visable end-product of the scanning operation is the NCI Grant Supported Literature Index, (formerly PARS Index) which is disseminated monthly throughout the NCI. The Index is a unique document representing the most complete record of accomplishments of the total extramural research programs. References are arranged by the grant number within program activities (e.g. cancer biology, immunology) and are also retrievable by subject in the accompanying keyword in context (KWIC) subject index. Original 3x5 cards produced at the time of scanning are prepared in multiple copies for Branch use and use by the Office for Cancer Communications. This permits the staff access to current material on a weekly basis prior to its publication in the Index.

The extent of effort and time involved in scanning a continually growing body of literature for references acknowledging NCI grant support and the production of information tools resulting from this operation have reached a magnitude warranting automation. Previously all listings and the Index were prepared on the Magnetic Tape Selectric Typewriter (MT/ST) necessitating multiple inputs of information and manual preparation of data such as hand-sorting of references. In September 1973 the assistance of the Division of Computer Research and Technology (DCRT) was engaged to prepare 6 tailored computer programs to produce the Index from input into a pre-existing text-editing WYLBUR system. Two programs produce the list of references by grant number within program area; two programs produce a comprehensive KWIC subject index; and two programs make history tapes for the list of references and KWIC subject index so that compatible data for automated retrieval will be available if the need should arise in the future. An IBM 2741 terminal was installed in October 1973 and actual use of the system began with the December 1973 issue of the Index. All 6 computer programs were completed by January 1974 and the system became completely operational in April 1974.

The advantages of automation of this function are manifold. It has overcome the necessity of multiple inputs of information and eliminated manual operations such as sorting. The saving in time here has made it possible to continue this operation despite the growing volume of material to be processed without increasing our staff. A second advantage is the capability to improve the currency of the Index which will appear monthly rather than sporadically. The new format of the subject index, keyword in context, would be impossible to produce manually. The in-depth indexing system, which is edited and augmented is one of the most important and needed additions to the Index for two reasons. First, the indexed word is qualified by the title in which it appears allowing a decision of acceptance or rejection of a reference without having to refer to the original reference. Second, with the increase in volume of information it is no longer functional to search large general categories for a

specific, but rather it is more expedient to start with the specific and proceed to the general only if necessary. Also new terms and concepts become apparent and are not lost in a free vocabulary system. Another advantage of automation is the compact storage of data on tape files plus the utility of storing information in a format compatible with automated information retrieval practices. The final advantage is a more professional appearance to the published Index. To finish off the new Index format, Capitol Systems Group, Inc., holders of a contract for support services to DCRRC, designed and supplied the cover.

Much progress has been made and it is hoped a more useful product is being supplied. However, there are many more improvements to be made. Additional areas under consideration for automation include verification of grant numbers and supplying of the principal investigator's name, compilation of a yearly KWIC index, and a search program to retrieve material from the history files. The extra function of scanning, previous to this year accomplished with outside assistance, is also under consideration as a potential function which could again be done in part through outside help.

During the year the follow-up survey of former NCI fellows and those trainees supported by Graduate Research Training Grants was successfully completed. This survey contracted with Westat, Inc. accomplished a 74 percent return rate of completed questionnaires for trainees and an 86 percent return rate for fellows out of the 3,816 person mail-out. From the 102 tables presenting data in graphic form, the findings were summarized depicting 66 percent of all trainees currently spending at least 10 percent of their time in research and 56 percent spending at least 10 percent of their time teaching or training others. For the fellows surveyed, 79 percent of all fellows are spending at least 10 percent of their time doing research with 68 percent spending at least 10 percent in teaching or training others in education institutions. The study shows that 79 percent of the trainees and 96 percent of the fellows have held a position in teaching and/or research since the end of their NCI-supported training. Additionally, when considering how many from these two recipient groups now spend 50 percent of their professional time on these activities, the results verify that the objectives of these programs have definitely been met. The Westat report also includes an analysis of the questionnaires according to: fields of training with emphasis on both M.D. and Ph.D. categories, additional degrees received, present employers, titles, cancer-related activities, sponsorship of others, publications, and most importantly the significance of National Cancer Institute's support with respect to its impact on their scientific careers.

In November 1973, the Westat contract was expanded to include some social implications of the Graduate Research Training Grants and Fellowship Award programs. Monroe Lerner, Ph.D., utilized twelve tables to draw conclusions with regard to the social implications of the programs' success. "...the central mission of the program, i.e., to provide research training where it might not otherwise be available, was successful. It seems clear that the vast majority of those for whom this training was provided continued to stay in research and developed their careers around it, thus providing the manpower pool for the extensive biomedical research effort conducted in this country."

The report of the follow-up survey results was distributed to NCI staff, appropriate National Institutes of Health staff members, and key members of the National Cancer Institute advisory bodies.

Presently the contract with Westat, Inc. is being broadened to include a study of about 300 former University of Rochester School of Medicine and Dentistry students, who participated in that school's Summer Cancer Training Program during the period 1961 - 1971. A control group of 300 medical students, who did not participate in this summer program, will be used for comparison purposes. The original questionnaire is slightly modified for this additional study. Of utmost importance to the entire program is the fact that the 300 students and 300 controls questioned can also be used as a control group in a comparative study with the NCI training and fellowship programs. To date no such control group has been available.

Since February the Branch has been involved in the development of one of the subsystems of the National Cancer Program - Management Information System (MIS) being developed under contract by the Systems Group of TRW, Inc. for the NCI. The Branch Chief has been named the Assistant Project Officer for this subsystem. The Scientific Indexing Subsystem is being designed to aid the Branch in analyzing the extramural programs, (grants and contracts) of the NCI and in preparing reports and responding to queries concerning these programs. The subsystem will contain administrative, geographic, and fiscal data as well as scientific data describing the nature of the research being performed. The subsystem will produce the fiscal year-end report "NCI Analysis of Extramural Programs" which provides statistics on major program research areas. Non-scheduled statistical reports can be requested involving any of the data in the system and providing: (a) number of grants and (b) amount of funding. Ad hoc queries specifying a combination of administrative and/or scientific criteria to be satisfied will provide specific information on individual projects, including principal investigator, institution, department, funding, program area, and descriptive scientific terms.

The Branch has been working closely with the Contractor to establish the requirements for the subsystem design. Periodic meetings have been held to discuss the files currently maintained, the current operating procedures, the reports produced, the types of questions received, and additional capability needed. The Contractor's design is being monitored during its development to insure that the Branch's needs will be satisfied.

OFFICE OF THE ASSOCIATE DIRECTOR
RESEARCH PROGRAMS

The Biomedical Research Programs Branch, the Clinical Investigations Branch, and the National Organ Site Programs Branch come under the direction of the Associate Director for Research Programs. The Associate Director is responsible for the effective administration and evaluation of ongoing programs in these branches. In concert with his program directors, and in consultation with active research scientists, he helps identify new approaches to program development in these areas.

BIOMEDICAL RESEARCH PROGRAMS BRANCH
CARCINOGENESIS PROGRAM

The Carcinogenesis Program is concerned with the achievement of a more effective prevention of cancer in man, caused or promoted by physical or chemical agents, principally the latter. In terms of the National Cancer Program (NCP), the Carcinogenesis Program contributes in varying amounts to the first six of the National Cancer Program Objectives. The primary classification of approximately 90 percent of the grants falls within National Cancer Program Objectives one through four, with about 80 percent of the grants clustered within Objectives one through three.

The Carcinogenesis Program has been composed of three major categories of grants: Chemical Carcinogenesis, Biochemistry of Cancer, and Cancer Related Biochemistry. The Chemical Carcinogenesis category consists of studies on the action and mechanisms of action of chemical and physical carcinogens, molecular structure-carcinogenicity relationships, the development of carcinogenicity screening procedures, and the properties of cells transformed by chemical carcinogens. Emphasis is on the mechanisms of action of chemical carcinogens, including such aspects as the toxicity, metabolism, and excretion of chemical carcinogens and their metabolites; the identification of proximate and ultimate carcinogens; factors which initiate, promote, or inhibit the action of chemical carcinogens; and biochemical changes in physiological compounds and processes, produced by chemical carcinogens. The Chemical Carcinogenesis category currently consists of 118 project grants for which the total costs amount to approximately $6.5 million. The Biochemistry of Cancer category focusses on the characteristic biochemistry of the cancer cell, the influence of cancer on the biochemistry of the host, and the development of biochemical and biophysical approaches to the early detection of cancer. The heaviest concentration is on studies dealing with nucleic acids and enzymes in cancer, and on endocrine related biochemistry of cancer and cancerous hosts. This category consists of 160 project grants, with total costs amounting to approximately $8.4 million. The Cancer Related Biochemistry category consists of biochemical studies on physiological compounds and processes which might provide or promote significant comparisons between malignant and nonmalignant states. The emphasis is on the biochemistry and biophysics of amino acids, proteins, nucleic acids and related compounds, and studies on the structure, synthesis, and activity of enzymes. In this category there are 62 project grants, with total costs of approximately $4.3 million. In future reports, Cancer Related Biochemistry grants will constitute a part of the newly established Tumor Biology Program.

The Chemical Carcinogenesis and Biochemistry of Cancer categories represent categorical research, whereas the Cancer Related Biochemistry category may be regarded as representing the noncategorical component of the Carcinogenesis Program. In this context, approximately 82 percent of the project grants and about 78 percent of the funds devoted to project grants in this program, now represent categorical research. The corresponding data for fiscal year 1973 were 77 and 73 percent, respectively.

Overall, the Carcinogenesis Program supports 340 project grants, 15 specialized centers, eight training grants, 27 fellowships, 14 research career development awards, and one research career award. The total cost of the program is approximately $32.0 million, distributed as follows: project grants, $19.2 million; specialized centers, $11.4 million; training grants, $0.7 million; fellowships, $0.3 million; research career and career development awards, $0.4 million. The total investment in research grants, consisting of both project grants and specialized centers, is approximately $30.6 million.

In this report, all references to cost are in terms of total costs, either actual or estimated. Of the project grants recommended for funding by the National Cancer Advisory Board at its March 1974 meeting, it is projected that about 50 percent will be funded; somewhat less than this estimate is included in the totals shown in the present report. It is expected, too, that the number of training grants and of fellowships to be funded during this fiscal year will exceed those herein reported.

Program Highlights

The work of Hollis et al. (CA 14817) contributes much needed perspective to the application of nuclear magnetic resonance (NMR) measurements to malignant tumors.

Spin-lattice (T_1) relaxation times were derived for nine mouse tumors, four human malignant tumors, and a variety of normal tissues. Measurements were also made on malignant cells maintained in culture. This is reported as being the most extensive study yet made on experimental animal tumors. Compared with normal tissues, T_1 values were found to be longer for all tumors, except one human sample. T_1 values for four mouse tumors grown in culture were above the range found for normal tissues and were comparable to the corresponding *in vivo* tumors. The cultured tissue samples (pellets) consisted of a hepatoma, melanoma, fibrosarcoma, and anaplastic carcinoma.

The human tissue types studied were lung adenocarcinoma, kidney squamous carcinoma, colon adenocarcinoma, and stromatosis. T_1 values for the latter three were clearly above the normal range. The T_1 value for the lung adenocarcinoma was not significantly different from that of the adjacent uninvolved lung. However, examination of the uninvolved host lung showed focal atelectasis, inflammation, and other abnormalities and, as such, could not be considered to be normal. This suggests that one of the requirements relative to the possible use of NMR in cancer detection, is knowledge as to whether a variety of pathologic conditions other than cancer might produce elevated T_1 values. It does not suffice, it seems, to limit the comparison to normal and obviously malignant specimens.

* * *

The diversity of carcinogens whose carcinogenic properties can be inhibited by the antioxidant, butylated hydroxyanisole (BHA), has now been extended (CA 09599) to include benzo[a]pyrene (BP), 7,12-dimethylbenz[a]anthracene

(DMBA), 7-hydroxymethyl-12methylbenz[a]anthracene (7-OH-DMBA), dibenz[a,h]-anthracene (DBA), uracil mustard, urethan, diethylnitrosamine, and 4-nitro-quinoline-N-oxide. Interest in the phenolic antioxidants, such as BHA, is based on their widespread use in foods prepared for human consumption; there arises the question of whether the consumption of such substances could affect the response to carcinogens. In the more recent studies, the addition of BHA to the diet of mice resulted in significant decreases in the numbers of pulmonary adenomas per mouse, associated with acute exposures to DMBA, BP, and uracil mustard. The DMBA and the BP were administered on two separate occassions by oral intubation; the urethan was administered as a single intraperitoneal injection; uracil mustard was administered on four separate occassions by oral intubation. Pulmonary tumor formation in mice was also inhibited by the addition of BHA to diets containing DMBA, 7-OH-DMBA, or DBA.

* * *

Work done under CA 13464 provides additional information concerning the possible relationship between UV-induced carcinogenesis and the photochemical conversion of cholesterol to cholesterol α-oxide, a carcinogen. The same workers had previously reported that cholesterol α-oxide is formed in vitro in human skin exposed to UV radiation. Exposure of albino hairless mice to suberythemic levels of UV radiation from a mercury arc lamp resulted in an increase in concentration of cholesterol α-oxide in the skin and this was associated with the subsequent appearance of squamous cell carcinomas. It appears that cholesterol α-oxide formation was not affected until after six weeks of chronic exposure to UV. The actual appearance of tumors occurred at a time when the level of the α-oxide had begun to decrease. It was found, too, that the in vitro formation of cholesterol α-oxide in mouse skin irradiated with ultraviolet light, can be inhibited by the use of skin derived from mice which had been kept on a diet supplemented with a mixture of antioxidants, consisting of ascorbic acid, D,L-alpha-tocopherol, glutathione (reduced form), and butylated hydroxytoluene. The supplemented diet was associated with an increased content of antioxidant in the skin and a decreased content of cholesterol α-oxide. The maximum increase in antioxidant (54 percent) occurred after two weeks of feeding, at which time complete protection against cholesterol α-oxide formation was observed. With continued feeding of the supplemented diet, the content of antioxidant decreased to about 18 percent above controls, at which time protection against the formation of cholesterol α-oxide was approximately 50 percent.

* * *

Several investigators have noted an inverse relationship between tumorigenicity and pore size of subcutaneously implanted Millipore filters, and it has been postulated that tumorigenicity is associated with an impairment of cellular content or communication caused by filters with the smaller pore size.

Foreign body tumorigenesis has been extensively investigated by Brand et al. (CA 10712) and now includes studies on the tumorigenicity by Millipore filters. Subcutaneous implantation of Millipore filters in the flanks of mice produced sarcomas at the implant site in 25 of 31 animals which had received filters with pore sizes of < 0.1 μm. Filters with pore sizes of ≥ 0.22 μm did not produce tumors. Histologic and ultrastructural studies performed at one, three, five, and ten months after implantation on specimens consisting of implant and surrounding tissue reactions, revealed that tissue reactions associated with nontumorigenic filters showed invasion of filters by macrophages or their cytoplasmic processes, as well as the presence of phagolysosomes within macrophages, as evidence of phagocytic activity. In tissue reactions associated with tumorigenic filters the above characteristics were not present; instead, there was more fibrosis, resulting in thicker collagenous tissue capsules. The hypothesis that the tumorigenesis observed with the use of Millipore filters with small pore size is associated with an interruption of physical contact between cells was not supported by this work, since 0.22 μm and 0.45 μm Millipore filters with a thickness of 150 μ did not induce tumors, while clearly preventing cell contact across the implant barrier. However, this does not exclude the possibility of a relationship between foreign body tumorigenesis and an interruption of diffusion-mediated cellular communication. Also, the data of the Brand group do not support the hypothesis that tissue anoxia due to poor vascularization is critical to Millipore filter tumorigenesis. They were not able to find any significant differences in the density of capillary network and depth of avascular zones in foreign-body reactions caused by tumorigenic and nontumorigenic filters.

* * *

In view of the critical importance of the development of approaches to the recognition of precancerous and early cancer states, it is of interest to note (CA 11736) the apparent ability to distinguish preneoplastic and neoplastic mammary tissues (mouse) from the normal gland, based on the levels of glucose-6-phosphate dehydrogenase (G6PD) isoenzymes. In this context, the hyperplastic alveolar nodule (HAN) is regarded as constituting a preneoplastic state. Normal mammary gland was found to contain two isoenzymes (G6PD-2; G6PD-3). Significant levels of a third, more rapidly migrating isoenzyme (G6PD-1) were found only in the preneoplastic and neoplastic mammary tissues.

* * *

A competitive inhibitor of aryl hydrocarbon hydroxylase, 7,8-benzoflavone, was found (CA 14661) to protect cultured rat liver epithelial cells against the cytotoxic effect of aflatoxin-B_1 and sterigmatocystin. Both of these mycotoxins are hepatocarcinogens. In these studies, the depression in acid-insoluble thymidine-^{3}H incorporation was used as a measure of carinogen-induced cytotoxicity. Estradiol-17B, shown by others to be a competitive inhibitor of mouse aryl hydrocarbon hydroxylase, was also found to be protective against the inhibitory effect of aflatoxin-B_1 on thymidine-^{3}H

incorporation. It was found, too, that the degree of protection provided was related to the concentration of estradiol-17B employed. As to other steroids tested, progesterone provided some protection, whereas testosterone and hydrocortisone showed little or no protective action. It was suggested that the protective action of estrogen against the cytotoxicity induced by aflatoxin-B_1 might be related to the reported, much higher incidence of primary liver cancer in men over women in parts of Africa and in Southeast Asia where aflatoxins, as contaminants in food, may play a part in the occurrence of the disease.

* * *

It has been shown that phorbol esters and other cocarcinogens inhibit DNA repair replication in normal human lymphocytes, and it has been suggested that such inhibition of DNA repair may consitute a general property of cocarcinogens (CA 12538). More recently, the phorbol ester, phorbol myristate acetate (PMA), was tested for its ability to inhibit thymine dimer excission in irradiated HeLa cells (CA 10978). These studies indicate that PMA inhibits the endonuclease-associated step of UV repair in HeLa cells and that this does not appear to be merely a toxic effect, since the plating efficiency of the cells was not affected.

* * *

Dr. Dao et al. (CA 04632) appear to have achieved an improved understanding of the effect of estrogen and that of prolactin in experimental mammary tumorigenesis. Increased release of pituitary prolactin greatly accelerated the growth rate of mammary tumors which had been induced in rats by the i.v. injection of DMBA (7,12-dimethylbenz[a]anthracene). Prolactin release was effected by the placement of electrolytic lesions in the median eminence of the hypothalamus. In these rats, ovariectomy was associated with a rapid regression of tumors in the presence of high levels of plasma prolactin. When a pair of ovaries was grafted into the ovariectomized rats with median eminence lesions, there occurred a rapid resumption of tumor growth without change in plasma prolactin levels. These findings would seem to indicate that prolactin-stimulated growth of mammary tumor requires the presence of ovarian hormones.

DRUG DEVELOPMENT PROGRAM

The Drug Development Program provides direction and support for development and evaluation of chemotherapeutic agents which act specifically or selectively against malignant growth with minimal toxicity to the subject host. The primary problems being addressed by the program effort relate to the continuing search for control, palliation and ultimate cure of cancer through therapy with more effective drugs and improved treatment programs. Outstanding investigators in various scientific disciplines are encouraged to pursue research in the chemical, biological and cellular aspects of abnormal as well as normal growth processes.

Total commitment in the Drug Development Program during fiscal 1974 was $22.70 million, representing an increase of $4.0 million over the previous year. Support of three hundred and seventy-two individual research grants amounted to $19.59 million. Three program project grants of broadly based fundamental studies in several biomedical disciplines involved $2.21 million. Additionally, $1.0 million was awarded to training projects, which include graduate training grants, research career awards and fellowships for development of potential research investigators. Fiscal resources are distributed among six primary component areas of the Drug Development Program in proportion to relative significance. The following summary shows research content and fiscal apportionment within the program categories:

1. Synthesis and Isolation: (178 grants, $8.58 million, 38 percent) Preparation of potential anti-cancer agents by synthetic chemical processes or by isolation from microbial, plant or animal sources; studies of structure determination and identification of known and potential chemotherapeutic compounds, particularly analogs of metabolic intermediates or aggregates. In 135 projects effort was devoted to the synthesis of compounds with novel chemical structures and analogs of compounds with established chemotherapeutic effectiveness in clinical trials. Forty-three grants involved isolation of potential anti-cancer agents from fermentation, plant or animal origin.

2. Preclinical Drug Evaluation: (68 grants, $3.51 million, 15 percent) Screening tests for anti-cancer activity, experimental therapeutics and toxicological and other pharmacological studies, criteria for selection of safe and effective dose regimens, countermeasures for toxicity. Effort in this area of drug evaluation complements the program of drug evaluation supported by the contract mechanism of the National Cancer Institute.

3. Mechanism of Drug Action: (119 grants, $7.06 million, 31 percent) Physiological disposition and drug metabolism, biotransformations, mode of action relating to antitumor and pharmacologic responses. Specifically, the mechanisms of several categories of drugs including nucleic acid analogs, folic acid antagonists, terpenes, alkaloids, antibiotics and glycosides, are being studied.

4. Clinical Pharmacology: (seven grants, $0.44 million, two percent) Pharmacologic and toxicologic studies in man through selected clinical investigations. Counteractive measures for toxic manifestations, preventive or supportive measures based on selective toxicity considerations are applied in clinical pharmacology for establishment of safe and effective dosage regimens for extended clinical trials.

5. Research Program Projects and Centers: (Three grants, $2.21 million, ten percent) Intensive thrust for total drug evaluation through broad multidisciplinary projects. These projects are given flexible support at the university departmental level for in-depth evaluation of problems in broad scientific disciplines. Preclinical as well as clinical elements are included in the investigations.

6. Research Training: (33 grants, $0.90 million, four percent) Research training support for graduate training, research career development program and fellowships, modified in conformance with Federal policy and directives pertaining to research training support in biomedical sciences.

Recent Accomplishments

Specific effort is directed toward investigation of various heterocyclic ring systems with bridgehead nitrogen atoms as anti-cancer agents. These novel structures containing various combinations of oxygen, sulfur and nitrogen atoms are interesting variants of the purine ring system which have shown promising chemotherapeutic activity. These include (a) practical laboratory syntheses of 3-substituted-3H-thiadiazolopyridines, pyrimidines, pyrazines and pyridazines to fused-ring systems where the 1,2,4-thiadiazole nucleus has been fused with the oxazole and 1,3,4-oxadiazole systems. These fused-ring systems have been synthesized for the first time; (b) studies on the use of ylide-type intermediates in the synthesis of bridgehead nitrogen, fused-ring systems; (c) use of 1,3-dipoles as reaction intermediates in obtaining structures not accessible by other routes. The synthetic procedures being developed by this study are capable of adaption to the synthesis of a wide variety of analogous products and consequently make available a wide variety of structurally novel compounds for pharmacological tests (Potts, Rensselaer Polytechnic Institute, CA 08495).

Special studies are being made to evaluate the prophylactic and chemotherapeutic potential of a number of ergot alkaloids in the control of murine mammary carcinoma, and to compare the effectiveness of a number of natural and synthetic ergot alkaloids in suppressing prolactin secretion. Recent findings have established that pituitary and ovarian hormones are among a variety of factors which influence mammary tumorigenesis in humans and in lower animals. Prolactin has been shown to be involved in this process. The recent discovery of the prolactin-suppressing activities of certain ergot alkaloids that appear to mimic the hypothalamic neurohormone PIF makes it possible for the first time to experimentally suppress prolactin with drugs.

These results suggest that prolactin is an important, perhaps essential hormone, in the developmental phases of mouse mammary tumorigenesis. Chronic suppression of prolactin secretion in young C3H/HeJ female mice virtually eliminated the appearance of mammary tumors. In older C3H/HeJ female mice, periodic suppression of prolactin secretion markedly reduced mammary tumor incidence.

Although the precise role of prolactin in human breast tumorigenesis is not entirely clear, sufficient evidence has been demonstrated for the singular existence of this hormone in man. There is a pressing need to define the relationship of prolactin to development and growth of normal, hyperplastic and neoplastic breast tissue (Welsch, Michigan State University, CA 13777).

Important contributions relative to synchrony of cell cycle and chemotherapeutic regimen have been made especially for the treatment of melanoma and squamous cell carcinoma of the cervix.

At low doses bleomycin reversibly inhibits cell progression at the S-G_2 boundary. Cells located in other stages of the cell cycle are essentially unaffected; therefore, when present for a complete cell cycle, bleomycin becomes a prospective *in vivo* cell-synchronizing agent. In five trials reported, bleomycin was used to synchronize human malignant melanoma cells *in vivo*. Tumor biopsies were pulse-labeled with tritiated thymidine and assayed by liquid scintillation and autoradiographic techniques. Following the synchrony block, cells at the S-G_2 boundary progressed to S phase in a partially synchronized wave. The labeling indices indicated about 1.5 to 4 times the normal number of cells in S phase at the peak times following the first bleomycin treatment. Therefore, bleomycin partially synchronizes cells *in vivo*, and this technique provides a rapid and accurate means for locating the synchronized cells and for scheduling a second drug for maximum effect on tumor cell killing.

The studies have demonstrated that in order for a drug to qualify as a synchronizing agent (whether *in vivo* or *in vitro*) the following criteria must be satisfied. The drug must produce cell progression delay at only one phase of the cell cycle. The delay must be reversible, preferably within a short time after the end of the treatment for synchrony. In addition, if the procedure is to be used *in vivo* to synchronize tumor cells in humans, the tumor should conform to certain criteria. The tumor must be accessible for biopsy and it must be large enough so that the process of multiple biopsies will not alter the kinetics of the tumor cells. The cell cycle time of the tumor in question should also be known since a synchrony treatment can be better scheduled if the cell cycle time can be considered.

It is technically feasible to obtain partial synchrony in human solid tumors and in leukemia cells. This technique may now be exploited in the treatment of these neoplastic diseases by well-designed chemotherapeutic regimens (Barranco, University of Texas, CA 15397).

A number of antibiotics alter the permeability of the membranes of eukaryotic cells. The ability of amphotericin B to increase the incorporation of certain drugs into fungi and animal cells with resultant enhancement of their effects has been exploited previously. The membrane-active antibiotics amphotericin B and polymyxin B enhanced the action of rifampicin, rifampicin analogs, and tetracycline against macromolecular synthesis and growth of mouse L-cells, human HeLa cells, and KB cells in tissue culture. The specificities of the second agents were maintained in that rifampicin inhibited RNA synthesis and tetracycline inhibited protein synthesis.

The concept that cellular integrity can be altered by a variety of drugs affecting the cell membrane is an important one. Its clinical usefulness will depend on the degree of selectivity that can be achieved so that only specific cell populations such as the malignant proliferating cells will be affected. There is evidence that malignant cells are more sensitive than normal cells to the action of polyenes, and preliminary animal experiments suggest that there is selectivity of the polyenes for populations of malignant cells. If this is the case, the synergistic combinations demonstrated in in vitro experiments will prove to have important therapeutic implications (Medoff, Washington University, CA 14656).

In the area of clinical pharmacologic studies of granulocyte infusions, advantage was taken of the setting provided by attempts to treat hematologic malignancy or marrow failure by allogeneic or isogeneic marrow transplantation. This procedure involves a predictably limited period of severe neutropenia in a situation where family members of known histocompatibility relationship to the patient may be used as granulocyte donors. Collections were made either by the continuous flow centrifugation method or by reversible leukoadhesion. Granulocyte infusion therapy was instituted and continued during the period between marrow ablation and the development of adequate peripheral function by the transplanted marrow. The decision to use granulocyte infusion support was determined by the presence of infection and by the availability of suitable donors and procurement facilities. Most patients were infected at the time granulocyte transfusions were started.

A program of granulocyte support was initiated in 18 instances. Fifteen of the recipients were patients undergoing allogeneic marrow transplantation from HL-A compatible sibling donors, and in three instances the marrow donor was an identical twin. All recipients achieved successful marrow engraftment except one who was being subjected to a second marrow transplant attempt after rejecting the first. The experience acquired in the management of infected patients using this technique has facilitated the design of a protocol for a prospective randomized controlled study to determine the value of prophylactic granulocyte support in uninfected patients with agranulocytosis (Thomas, University of Washington, CA 10167).

Administration

The overall funding level and direction of research effort in the basic components of the program will continue to be maintained in conformity with the goals and objectives of the National Cancer Plan. The scope and relative emphasis in individual program categories will be adjusted as necessary and as new findings and significant developments are recognized in the research effort. The total level of research support in the Drug Development Program increased approximately 25 percent over that of the previous year. Appropriate adjustments of program priorities have been maintained within the fiscal allocations through careful assessment of the need for intensive research effort in individual component categories of the program.

The organizational structure of the program entails supervisory functions in the scientific and fiscal management of grant supported research. The Program Director, with the aid of one grants assistant, maintains full surveillence and ensures coordination of scientific aspects of the total program. The fiscal budgetary and management aspects are coordinated through a parallel staff structure in the Grants Administration Branch of this Division

Future Plans

The key problems in successful chemotherapy relate to cell population kinetics, precise assay of tumor cells, pharmacokinetics, supportive measures to counteract toxicity and development of predictive test systems, especially for slow growing solid tumors. Most drugs currently in use were derived from empirical screening in mouse tumors with a high rate of proliferation. New test systems are being introduced to simulate the slower growth rate of their clinical counterparts, such as carcinomas of the lung and breast. Greater emphasis will be placed on test models involving spontaneous virus induced tumors such as AKR leukemia and C3H mammary cancer.

There is a need for development of more precise and sensitive means for determining the amount of residual tumor during progressive stages of treatment. Current methods for assessment of disease and prognostic evaluation are relatively inadequate. Present knowledge in this area focuses on such parameters as polyamines, methylated tRNA degradation products, CEA, α-Fetoprotein, Regan's isoenzyme and others. The need for quantitative knowledge of the pharmacologic disposition of anti-cancer agents and the problems of drug-receptor and drug-disease interactions as they affect both antitumor activity and host toxicity, have become more clearly appreciated.

An important consideration in the design of cancer chemotherapy is the selective toxicity which, in part, depends on key differences between the amount, protein nature and activity of potential key enzymes that play a crucial role in the growth process. A study of the mechanisms controlling gene expression in normal and neoplastic cells should reveal specific points of attack to exert control over the function and proliferation rate of cancer cells. These areas will remain the targets of intensive effort in future research.

EPIDEMIOLOGY PROGRAM

The grants program in Cancer Epidemiology consists of studies of cancer epidemiology and human population genetics; biometry and biomathematical technology applied to cancer research; biomedical communications and psychosocial factors. The major emphasis in fiscal year 1974, as in previous years, has been in epidemiology, using demographic, laboratory and clinical measures to define the probable contributions to human malignancies of factors such as viral infections, carcinogenic chemicals, radiation exposure, endogenous and exogenous hormones, genetic predisposition. Since biometrics and biomathematical analyses are used in defining the probabilities that one or more of these agents cause cancer, research concerned with improved analytic and biomathematical techniques is naturally related to the epidemiology program. Ongoing studies are concerned with such topics as improved techniques for epidemiologic analysis; modeling of tumor behavior under varying conditions of treatment or genetic or biologic environment; and the development of computer technology to augment cancer research. Similarly, psychological and social variables are among the demographic measures which are useful in defining similarities or differences between persons susceptible or not susceptible to cancer. Research is concerned with the effectiveness of intervention as well as defining characteristics which cancer patients have in common.

While biomedical communication projects are assigned to this program primarily for administrative reasons, two of the three are concerned with standardizing disease classifications, a quality control factor of great importance to epidemiologic studies.

Program Statistics and Administration

In fiscal year 1974, the total number of research grants in the Epidemiology Program increased from 33 to 39. Grants concerned with training increased from three in 1972 to six in 1974. Funds committed to the program increased from $4.004 million in fiscal year 1973 to $5.246 million in fiscal year 1974. During the year, the number of epidemiologic projects increased from 20 to 24, studies of psychosocial factors increased from two to four, biomedical communications projects from one to three. Biometric studies decreased from eight to six and the number of genetic studies continued to be two.

In accordance with a trend since 1971, the proportion of the research supported by regular research grants increased in contrast to program project support. The number of program projects (seven) remained the same although one is being phased out. This may be of some significance since publications and progress reports from the program projects indicate that research is moving rapidly into new and less well documented areas consistent with the National Cancer Institute's recent interest in organ site programs. Among the regular grants, there is a tendency toward the application of sophisticated analytic techniques to define the role of factors now known to be associated with malignancies, such as viral infections in relation to breast

and cervical cancer, hormones relative to breast cancer, viruses relative to lymphomas. There are few projects concerned with the epidemiology of colon, lung, bladder or prostate cancer. This probably reflects the success of the organ site programs in attracting appropriate epidemiologic studies, but further evaluation seems indicated.

An area requiring further exploration concerns the disapproval rate and subsequent fate of grant applications assigned to the epidemiology program. The overall disapproval rate exceeds 62 percent. Very modest follow-up inquiries indicate that some applicants for whom disapproval was recommended by research grant study sections have attracted funding by contract for similar studies (e.g., a New Mexico study of cervical cancer).

Study sections cite poor quality and inadequate investigator preparation as reasons for the high disapproval rates. In an effort to correct these deficiencies, two steps were taken during fiscal year 1974.

First, a special need for training in cancer epidemiology and biometry was recognized by the National Cancer Institute, the National Institutes of Health, and the Department of Health, Education and Welfare, resulting in a new cancer research fellowship program beginning in fiscal year 1975 (Postdoctoral Institutional Research Fellowship Award). The new program should provide expertise in both cancer research and in epidemiologic methodology. Discussions among cancer scientists, clinicians and epidemiology faculty were initiated during fiscal year 1974 to stimulate collaboration in epidemiologic and biometric training.

Secondly, in collaboration with cancer centers, program goals for epidemiologic/statistical programs in cancer centers were identified and a guideline statement was drafted. Such programs should provide improved data for epidemiologic studies and improved interaction among basic scientists, clinicians, and epidemiologists.

These two actions provided the groundwork for a long-range program of expansion and improved quality for cancer epidemiology and the closely allied areas of biometry, biomathematics and computer sciences, human population genetics and research into behavioral factors associated with cancer. The development of epidemiologic and biometric expertise in specialized cancer centers should result in an improved range of research topics.

Program Highlights

Dr. Marvin Zelen's analysis of randomized clinical trials for lung cancer (CA 10810) has revealed that the Karnofsky Performance Scale is the most important prognostic variable which correlates with survival. This finding has immediately been incorporated into the planning of lung cancer clinical trials throughout the world.

Another study with new findings of potential significance for health practices was reported by Drs. Newell (1 K04 CA 70116-01), Rawlings, Kinnear

and Correa from New Orleans working in collaboration with Drs. Henderson, Dworsky, Menck, Thompson and Sheehan from Los Angeles, California. Using the same methodology in the two geographic areas, cases of Hodgkin's disease and controls matched for age, sex, race and socio-economic status were surveyed for historical and serologic data. Spouses and siblings were also used as controls. Neither tonsillectomy, appendectomy, nor prior infectious mononucleosis were confirmed as risk factors but prior use of dexedrine (amphetamine) may be a significant risk factor for nodular sclerosis and mixed-cellularity types of Hodgkin's disease. Further studies are indicated in view of the widespread practice of treating hyperkinetic children with amphetamines.

The epidemiology of breast and cervical cancers continues to intrigue many well-qualified investigators. The finding that the incidence of breast cancer is higher among women in higher socio-economic levels whose first pregnancy occured late in life relative to the onset of menses has focused interest on the relationship between hormones and breast cancer. Factors currently under investigation among cases and controls include changes in estrogen ratios associated with childbirth; variations in estrogen patterns associated with irregular early menstrual history and with relative infertility; and the effects of hormones administered therapeutically or to modify fertility. The chemical composition of breast secretions, the genetic characteristics of women of mixed racial derivation, the prevalence of viral markers are all being investigated in breast cancer case-control studies. In recent months, the relationship of diet to breast cancer susceptibility is of increasing interest as are possible relationships between risk of breast and of colon cancer and between cancers of the breast and those of the endometrium and ovary.

Cancer of the uterine cervix is also a popular topic of investigation. Two retrospective studies, Dr. Julius Schachter (CA 14427) and Dr. E. Russell Alexander (CA 11703), are directed toward the role of viral infections (including Type 2 Herpesvirus cytomegalovirus and Chlamydiae) in cervical dysplasia, carcinoma in situ and invasive carcinoma. These two studies as well as one reported by Dr. David Thomas (CA 11489) suggest that viral infections are more frequently associated with cervical dysplasia than with either carcinoma in situ or invasive carcinoma. Dr. Thomas finds trichomonas infections more frequent among cervical cancer patients than herpesvirus infection. The detailed prospective study of cervical dysplasia is emerging ever more clearly as a key step in understanding cervical cancer.

Dr. Arthur Herbst (CA 13139) continues his extensive study of clear cell adenocarcinomas of the vagina and cervix in offspring of women who received nonsteroidal estrogens during the first five months of gestation. He observes that cervical adenocarcinomas, while less frequent, seem to be more virulent with earlier spread to regional lymph nodes than vaginal malignancies. Neither type is reliably revealed by cytologic studies or symptoms. He is also following 143 individuals without cancer who are known to have been exposed to maternal estrogens during gestation. Approximately 30 percent display gross evidence of adenosis subsequently

proven by biopsy and 10 percent have congential vaginal malformations. A pilot study of the effectiveness of vaginal suppositories of progesterone in physiologic dosage has shown enough effect in clearing inflammation and decreasing adenosis that a double blind study is now underway. This study should be of great importance for the follow up and management of individuals at risk now being identified through a national network of studies sponsored by the Cancer Control Program.

Genetic studies are providing some interesting clues for further investigation. Dr. Nicholas Petrakis' study of genetic distance among women with breast cancer (CA 13556) suggests that Negro women become more susceptible to breast cancer as the proportion of Caucasian admixture increases. Meanwhile, Dr. Michael Swift (CA 14235) has been studying the malignant experience among relatives of persons with ataxi telangiectasia who are known to be at high risk of neoplasia. His preliminary findings suggest that relatives are also at higher risk than the general population for various types of malignancies, and especially those involving the pancreas, gall bladder and bile ducts.

CANCER BIOLOGY PROGRAM

The Cancer Biology Program is designed to provide fundamental information on the causes and nature of cancer in man, with the expectation that this will result in better methods of diagnosis, prevention, and treatment of neoplastic diseases. The program comprises the areas of Viral Oncology, Tumor Biology, and Cancer-related Biology.

Viral Oncology is concerned with the role of viruses in the etiology of cancer and the consequences of this for identification of susceptible individuals, early diagnosis, and development of new modes of prevention and treatment. Investigations include studies at the cellular and molecular levels as well as the animal level. Research on the mechanism of viral oncogenic transformation and the significant phenotypic expressions resulting from transformation occupy a major aspect of the program. Investigation of gene expression and cell regulatory functions, utilizing viruses as tools, are also an important part of the program.

In Tumor Biology the emphasis is on the nature and properties of the preneoplastic and neoplastic lesion, on the tumor-host relationship in its various manifestations, and on the biology of the tumor cell.

Cancer-related Biology encompasses aspects of cell biology, molecular biology, embryology, genetics, morphogenesis, pathology, and other disciplines that may range beyond the confines of manifest cancer research, but contribute significantly to it, conceptually and experimentally. While the greatest interest resides in the biology of animal, including human, cell systems, projects which involve other forms of life, if these offer particular advantages, are also considered to be appropriate for the Cancer Biology Program.

During fiscal year 1974, the Cancer Biology Program consisted of 347 grants totaling $32.0 million. These included 331 traditional projects ($22.0 million) and 16 Program Projects and Basic Science Centers ($10.0 million). One-hundred-fifty-four of all grants in the program ($15.0 million), were in Viral Oncology, 123 ($12.5 million) were in Tumor Biology and 69 ($4.5 million) in Cancer-related biology.

* * *

Highlights

Viral Oncology continues to move along on a broad front providing new information and ideas in oncology, molecular biology and medicine.

A promising approach in the analysis of the genome of the transforming DNA viruses has developed at Johns Hopkins University, (Nathans, CA 11895). Enzymes ("restriction" endonucleases) have been isolated from bacteria, especially H. influenzae, which cause specific, reproducible fragmentation of SV40 DNA. The position of the fragments in these genomes has been determined and the function of the sequences are now being investigated.

An important goal of this type of investigation is to identify the transforming gene of the virus and the nature of its interaction with the genome of the cell. In productive, non-transforming infections, the genome of the resulting virus may occur combined with varying amounts of cellular DNA. Nathans and his colleagues have found that particular sequences of cellular DNA are integrated at specific loci of the viral genome, offering a possible clue of where the viral genome is inserted in the cell genome in transformation. This seminal research has stimulated a hunt for other restriction enzymes (Sharp et al, CA 11432) and applications to other types of DNA (Petterson et al, CA 11432).

Even with as small a virus as polyoma or SV40, whose genomes can code for only about six or seven average size proteins, it is difficult to distinguish viral gene products from secondary effects in the perturbed cell. The value, therefore, of cell-free viral systems to synthesize discrete proteins is obvious. Such a system has been developed by Cranford and Gesteland at Cold Spring Harbor (CA 11432), and represents the first example of protein synthesis in a cell-free system directed by a mammalian virus DNA, in this case polyoma.

The papova viruses, particularly SV40, are valuable models for basic biological and molecular studies in oncogenesis, gene expression and cell regulatory processes because of the small size of their genome, their relatively simple structure and ease of experimental manipulation. Agents of this type, however, are of more than academic interest. They are currently attracting much attention in clinical medicine because of the association of papova viruses related to SV40 with progressive multiple leuccoencephalopathy, kidney transplantation, and brain tumors. Some of these agents have been found to be transforming and oncogenic in hamsters (Shah, CA 13478). This field should expand markedly in the next few years.

In the herpes field, George Miller (CA 12055) at Yale University has made the very significant observation that Epstein-Barr virus (EBV) induces malignant lymphoma in marmosets. This finding adds a new dimension to the role of this virus in Burkitt's lymphoma and raises anew the question of the significance of infectious mononucleosis, which is almost certainly caused by EBV, (Henle, CA 4568), in human malignancy.

Another finding with public health implications is reported by Robert Marshak of the School of Veterinary Medicine, University of Pennsylvania (CA 14193). This group has been studying the biology and natural history of bovine leukemia, in which a "C type" (i.e. RNA tumor-type) virus is implicated. Two of six chimpanzees fed since birth on untreated milk from infected cows died with leukemia at about eight months of age. It is noted that leukemia has never before been reported in this primate.

* * *

The RNA tumor viruses are extraordinarily efficient oncogenic agents in vivo, and the transforming types are capable of rapid and strong transformation in vitro. They are much more complex in structure relative to the papova viruses and, unlike the latter, generally replicate in the transformed cells, constituting a disadvantage in certain types of oncogenesis research. These agents are widespread in nature and causal factors in naturally occurring leukemias, lymphomas, sarcomas in divers species and of carcinoma, at least in the mouse. The expectation, therefore, is that similar viruses are implicated in human cancer. This has yet to be convincingly demonstrated. In various vertebrate systems--such as the mouse, cat, fowl and others--this line of research has been enormously productive of new information in the molecular biology of the cell and the nature of the cell surface, and has contributed substantially to the development of sophisticated methodology in genetic chemistry.

Among recent results obtained by these techniques is the demonstration of DNA transcripts of RNA tumor virus reverse transcriptase covalently linked (integrated) with the genome of transformed cells (Bishop, CA 12705). While integration may not be limited to transforming oncogenic viruses, it is almost certainly a necessary first step for transformation. That integration of a complete transcript of an RNA tumor virus may occur is indicated by the recovery, in some instances, of infectious DNA (provirus) from such cells (Temin, CA 15149).

Integration is well known to occur in the case of DNA tumor viruses. A new finding (Butel, CA 15149) is the recovery of infectious DNA from such transformed cells, a notable achievement considering the very small amount of integrated viral DNA buried in a vast amount of cellular DNA, again attesting to the power of modern molecular techniques.

Possibly the most intriguing concept to arise recently in the RNA tumor virus field concerns the non-transforming and possibly non-oncogenic C type viruses resident in normal cells--the so-called endogenous or "zenotropic" viruses (Levy, CA 13086). Generally, they are unable to replicate in the cells of the natural host but may in those of a foreign host. Their ubiquity, especially in foetal tissue, the ease with which they can be induced to appear by various manipulations in cells otherwise lacking all vestige of virus by the most sophisticated tests, suggests the possibility that they play some normal physiological function, perhaps in cell differentiation (Koprowski, CA 10815) or in the transfer of genetic information (Vogt, CA 13213).

Treatment of mouse melanoma cells with low levels of bromodeoxyuridine does not significantly affect cell growth, but may markedly reduce their oncogenicity (Silagi, CA 10095). A similar effect occurs with cells of the Friend disease in response to dimethyl sulfoxide (Friend, CA 10000). Silagi attributes the effect she observes to enhanced immunogenicity of the treated melanoma cells; these effects--reduced oncogenicity and enhanced immunogenicity--are accompanied by the secretion of budding C

type viruses from the cell surface. Dr. Friend also notes a marked increase of C type particles in Friend cells treated with DMSO. The question raised by Silagi is whether budding C type particles play a role in the enhanced immunogenicity of the cells by altering the plasma membrane during the budding process. Todaro (Biohazards in Biological Research, Cold Spring Harbor, 1973) similarly suggests that endogenous viruses in their natural host may conceivably protect against cancer, though they may be infectious and even oncogenic in a foreign host.

* * *

Fidler (CA 14256) devotes himself to the important problem of metastasis. As a model he used B16 melanoma cells intravenously in C57 BL/6 mice and quantitates lung metastases. In this system he finds that 1×10^6 lymphocytes, when incubated with melanoma cells, enhances metastasis, whereas 1×10^7 reduces it. This study illustrates the delicate balance which determines the fate of a tumor. For this and other reasons (George Moore, CA 15185) a cautious approach to the use of lymphocytes as a therapeutic measure in cancer is indicated.

* * *

It need hardly be said that numerous other advances have been made with the support of grant funds allocated to the Cancer Biology Program. Many of these are described elsewhere in this report.

* * *

Administrative

In consideration of the growth and increasing complexity of the Cancer Biology Program, the following reorganization will take place: Viral Oncology, to include cancer virology and related studies, will be a separate program with Dr. Philip G. Stansly as Program Director. Tumor Biology will be headed by Dr. George M. Willis and will include tumor specific biology and cancer-related biology and biochemistry.

CLINICAL INVESTIGATIONS BRANCH

As outlined in last year's report, the Clinical Investigations Branch continues to function as the organizational focal point for grant-supported therapeutically oriented research programs.

The present programs of the branch are:

Cooperative Groups
Supportive Care Research
Radiation
Chemotherapy
Immunology
Diagnostic Research and Prevention
Surgery

Detailed reports for each program are presented below.

Although the Radiation Branch of the Division of Cancer Biology and Diagnosis carries out an extensive intramural program, there is no collaborative contract-supported activity elsewhere in the National Cancer Institute comparable to the parallel programs in carcinogenesis, pharmacology, or viral oncology, supported extensively by both grant and contract. The Radiation Program is unusual in that it constitutes the majority of NCI-supported activity in the area of radiation therapy. With this in mind and because a large component of current therapy for cancer nationwide is provided by radiation therapists, it is of great importance to maintain the strength of the Radiation Program of the Division of Cancer Research Resources and Centers in the future.

Throughout the Branch, program budgets have undergone substantial increases in the last few years (as much as 200 percent in some instances). However, increased personnel support for these programs has lagged far behind. Most program staff is currently hard-pressed to provide the minimal amount of administrative effort necessary for program maintenance. Under the new National Program guidelines, interdivisional coordination of programs receives a high priority. Because they are the persons most familiar with program scientific content, the current Program Directors are the ones most likely to effectively implement the task of coordination. Additional personnel are therefore crucial if these responsibilities for increased coordination are to be met.

Yet another consideration regarding the needs for additional personnel is that the maximum amount of research effort at the least management cost to the NCI is achievable through the grant-supported programs. The record seems clear in this regard. Nevertheless, to take advantage of this economic phenomenon, program staff personnel increases will be mandatory.

COOPERATIVE GROUPS PROGRAM

Components of the Program

In addition to the 19 clinical cooperative groups active at the beginning of this year, several new groups were recommended for approval and began their grant-supported activities this year. The ongoing, fully active groups are:

Acute Leukemia Group B
Central Oncology Group
Children's Cancer Study Group
Cooperative Breast Group
Eastern Cooperative Oncology Group
Gynecologic Oncology Group
National Wilms' Tumor Study Group
Polycythemia Vera Study Group
Primary Breast Cancer Group
Radiation Therapy Oncology Group
Radiotherapy Hodgkin's Disease Group
Radiotherapy Prostatic Cancer Group
Southeastern Cancer Study Group
Southwest Oncology Group
Surgical Adjuvant Renal Cell Group
Veterans Administration Urological Group
Western Cancer Study Group

The Radiotherapy-Chemotherapy Head and Neck Cancer Group continues to function, but is now a constituent of the Radiation Therapy Oncology Group, maintaining a degree of separate identity but no longer reviewed and funded separately. The Preoperative Irradiation Lung Cancer Group continues its long-term follow-up function and does not plan to activate new protocols.

The three developing groups are:

Malignant Melanoma Clinical Cooperative Group
Mycosis Fungoides Study Group
Diagnostic Breast Cancer Group

The first of these is based upon dermatology as the primary discipline of the membership; also included are histopathologists, surgeons, laboratory scientists, and an epidemiologist. The activities of this group are centered on development of standard procedures for the study and evaluation of early primary cutaneous melanoma.

The second group has made considerable strides toward agreement on standardized and histopathologic criteria for a staging scheme in mycosis fungoides. The third group continues work on the development of their planned study of the usefulness of mammography for serial screening of women at high risk for developing breast cancer.

The Veterans Administration Cooperative Urology-Radiology Research Group will receive initial support in fiscal year 1974 for finalization of detailed plans for group studies and for a radiotherapy treatment workshop meeting.

The steady increase in studies of both Hodgkin's and non-Hodgkin's lymphomas has resulted in a progressively expanding workload for the Lymphoma Pathology Panel. The general acceptance of the importance and usefulness of this resource function, supported through the Branch, is gratifying.

A histopathology workshop of international scope was held under the auspices of the Chairman of the Lymphoma Pathology Panel in October 1973. Considerable increase in uniformity of histopathologic classifications of the non-Hodgkin's lymphomas developed as a consequence of this workshop. The subsequent effects of this classification will be explored as the various groups add this dimension to their studies in the forthcoming year.

With the increasing number of protocols which include radiation therapy as a portion of the treatment, the workload of the Radiological Physics Center, another grant-supported service activity, has increased also. This center monitors the radiation therapy source output estimates and the dosimetry calculation methodology for each institution participating in these studies. It is developing the capacity for retrospective analysis of treatment parameters for every patient in many of these studies, a quality-control function believed crucial to valid comparisons of results.

Intergroup collaboration on common protocols has increased during the past year. The Eastern Cooperative Oncology Group and the Primary Breast Cancer Therapy Group are conducting a joint study of postoperative surgical adjuvant chemotherapy in high-risk groups following radical mastectomy. The Central Oncology Group and Radiation Therapy Oncology Group are collaborating in a common protocol to evaluate the usefulness of preoperative irradiation in patients with operable colorectal carcinoma. The Eastern Cooperative Oncology Group and Southeastern Cancer Study Group continue their fruitful collaboration in studies of multiple myeloma. Intergroup studies of Ewing's sarcoma and rhabdomyosarcoma, initiated by the three groups with significant potential for the study of pediatric solid tumors--Acute Leukemia Group B, Children's Cancer Study Group, and the Pediatric Division of the Southwest Group--are under way.

All the above-noted developments are indicative of two major trends--the increasing involvement of laboratory scientists in clinical studies and the greater extent of both interdisciplinary and intergroup cooperation. Both trends are highly desirable and will be vigorously fostered.

Meetings Sponsored

In addition to the regularly scheduled Group Chairmen's Conferences and Group Statisticians and Data Managers meetings noted above, the conduct of scientific symposia continues to be an important aspect of the Branch's activities.

A clinically oriented open symposium on myeloma was held October 22-23, 1973. Proceedings will be published in the Archives of Internal Medicine. This meeting will represent the definitive myeloma meeting for the next few years.

A Symposium on Combined Modality Therapy for Cancer was held in Denver in February 1974. This was an open meeting, and the proceedings will be submitted to Cancer for publication.

This Cooperative Group Program is the most extensive program of its type in existence with a clear central focal point. The groups in this program include about 2000 physician investigators who annually study around 20,000 patients on over 350 clinical protocols employing all three anticancer modalities--surgery, radiation therapy, and chemotherapy--singly and in combination.

The average group in this program includes 20 institutional memberships consisting of about 100 investigators who study about 1200 patients a year on 18 to 20 different protocols. The data generated from these studies require the services of a fulltime professional biometrician and several data management experts for proper handling and analysis. There are three meetings a year of the whole group, and a number of working committee meetings as well, usually in association with a major scientific gathering. The minutes include a detailed status report for each protocol, in addition to reports of the whole group and subcommittee deliberations and statistical reports regarding each institution's contributions.

Monitoring of the scientific aspects of group activities is an important component of staff work in this Program, as is liaison and coordination between this Program and the Division of Cancer Treatment, the Breast Cancer Task Force, DCDB, the National Organ Site Programs, DCRRC, the Committee on Controlled Clinical Trials of the International Union Against Cancer, and the European Organization for Research on the Treatment of Cancer. Each of these organizations has clinical trial programs under way, and the liaison effort is conducted in the interest of avoidance of unnecessary duplication of clinical research efforts. As part of this activity, copies of each group's minutes (20 groups times three meetings a year) are sent to each of the other Group Chairmen, to the Cancer Therapy Evaluation Branch, DCT, and to the UICC and the EORTC. Annual group progress reports are similarly distributed. Program staff must be steadily aware of the ongoing trials and the groups' performances therein in order to monitor the overall operation for appropriateness and effectiveness, as well as to make the various liaisons useful. As can be imagined, continually keeping up with this data input is a major task.

In the interests of improved intergroup cooperation and to provide forums for joint consideration of individual solutions to mutual problems, a one-day conference of all Group Chairmen is held annually, and a two-day combined Group Statisticians and Data Managers Conference is held semiannually. All the arrangements for these conferences are made by the Program staff, including preparation and distribution of minutes of the scientific proceedings of each Conference.

Pharmacology support units, developed by two of the cooperative groups, are now operational. These units provide the involved groups with in-depth laboratory capacity for carrying out studies of biochemical pharmacology and pharmacokinetics on drugs either currently in use in group studies or as developmental investigations prior to initiating a group clinical trial. It is expected that at least one more group will activate such a unit during the coming year.

International Liaison

As a consequence of some of the activities of the U.S.-U.S.S.R. international cooperation with regard to cancer, requests for information regarding the structure and function of the Cooperative Clinical Trials Program have come from several key Russian centers. The Russian government-run medical care system is, in many respects, ideally suited to the development of a program similar to this, and it is hoped that further developmental efforts may be fostered through this mechanism.

The degree of liaison between this Program and the European Organization for Research on the Treatment of Cancer (EORTC) continues to expand. NCI support has been provided for a central statistical and data management office for the many EORTC cooperative group studies. We look forward during the next year to more effective trans-Atlantic cooperation in clinical studies at the functioning cooperative group level in addition to the program headquarters level.

SUPPORTIVE CARE RESEARCH PROGRAM

This Program is directed towards developing the necessary knowledge to permit 1) long-term control of hemorrhage by successful, repeated platelet transfusion, and 2) control of infection by leukocyte transfusion to remove susceptibility to infection and by the use of the Protected Environment (sterile laminar air flow rooms) to keep the susceptible patient from becoming infected. At present under this Program five grantees are carrying out studies of both platelet and leukocyte transfusions, 15 institutions are doing platelet research, and two Protected Environment units are in operation. The total budget for this Program in fiscal year 1974 is $1,574,000. In December 1973 a working conference was held of grantees in the Supportive Care Research Program for presentation of the latest results and of work in progress regarding platelet and white cell transfusion.

Although this is not a large program, there is a clear need for close surveillance of the studies being done for several reasons. First, this is costly research, both as regards the unique biological resources of human platelets and leukocytes used and as regards the other costs of the research in relation to the numbers of patients which can be so studied. This latter is particularly true for Protected Environment studies. Second, the pressing need for successful results in clinical practice justifies close scrutiny to avoid any delays in the process of translation of research results to patient care. Third, there are sizable programs with similar goals under way under the auspices of the Division of Cancer Treatment, NCI, and in the National Heart and Lung Institute. Close liaison with these other programs is essential. However, personnel restrictions have not permitted the acquisition of a fulltime Director for this Program. It is hoped that in fiscal year 1975 this serious deficit may be remedied.

RADIATION PROGRAM

The Radiation Program is concerned with the full spectrum of radiation research, from radiation physics and chemistry on one hand, to radiation biology and therapy on the other. The Program's ultimate goal is the improvement of radiation therapy as a cancer treatment modality.

Because of the unusual scientific breadth of the Program, it has proved effective to commit its execution to two program directors acting in a complementary fashion, one for Radiation Therapy and one for Radiation Biology and Physics. The former position is traditionally filled on a rotating basis by an academic radiotherapist on sabbatical leave; the latter by a permenant health scientist-administrator.

Program activities are supported by traditional research grants, exploratory or planning grants, basic and clinical research center grants, grants for cooperative clinical studies, and a small number of research contracts and interagency agreements.

In the last fiscal year the radiation program has undergone vigorous growth especially in traditional grants and high LET center grants, the former increasing by 45 percent to $5.0 million and the latter by 100 percent to $4.0 million. At the present time and for the foreseeable future the high LET effort can clearly be considered the top priority thrust of the Radiation Program. It is hoped that its funding level will again double in fiscal year 1975 and for the subsequent three years increase at $4.0 million per year.

Widespread excitement has been generated in recent years in the radiation therapy community based on the possibility of a quantum jump in cancer cure as a result of new high LET (linear energy transfer) radiation therapy modalities. Such modalities include neutrons, pions, and stripped nuclei. Reductions in the annual national cancer death rate as great as 15-20 percent have been hypothesized in some circles. Even far more modest reductions would be considered revolutionary.

The promise of high LET radiation therapy is an excellent example of the growing contribution of radiation biology and radiation physics to radiation therapy. Research in these basic disciplines has generated the groundwork for this prospective, new clinical modality. Indeed, the postulated advantages of high LET radiation therapy can be resolved into physical ones and biological ones.

Physical advantages relate to the possibilities of higher tumor doses coupled with lower transit doses and of high tumor dose with low dose to nearby, highly radiosensitive normal tissue. A number of potential biological advantages have been suggested. Among them are the possibilities of reduction in the radioprotection of many tumors because of their hypoxic state and of reduction of radiation damage repair in tumors versus healthy tissue. Various types of high LET radiation have various mixes of these advantages. Neutrons are expected to have only biological advantages; protons, only physical advantages. Pions and heavier ions are expected to have both in different proportions.

At present, the radiation program is supporting three neutron efforts, one pion effort, and one heavy ion effort in various stages of development toward randomized clinical trials. The three neutron efforts are all in the human radiobiology stage, having treated approximately two hundred patients. It is expected that a cooperative randomized neutron trial will get underway in fiscal year 1975. The pion effort is in the preclinical radiation biology and physics stage, with plans for human radiobiology for fiscal year 1975 and a randomized clinical trial for fiscal year 1976. The heavy ion effort is in the preliminary radiation biology and physics stage.

Grant proposals will soon be forthcoming to expand the neutron and pion efforts, to get a proton trial underway, and to design and develop hospital-optimized high LET radiation generators. The program is now at the stage where comparatively modest infusions of funds will significantly reduce the time to a definitive assessment of high LET radiation therapy.

CHEMOTHERAPY PROGRAM

The Chemotherapy Program is completing its third fiscal year of activity as a section of the therapeutic modalities programs of the Clinical Investigations Branch. In fiscal year 1974 the program budget was $4,295,000 as compared to $477,697 in fiscal year 1972 and $2,414,340 in fiscal year 1973. These increases have allowed reasonable expansion in the Program. Further growth is necessary and expected to ensure that all possible areas in chemotherapy not yet fully studied are completely explored.

Areas of future emphasis will be in combined modality clinical trials, pharmacokinetic studies, the role of blood coagulation in malignancy, and studies on the protection of normal tissue during treatment. Additional funds must be available to the program for these new explorations. The growth of the program should accelerate in the near future as the Centers Program adopts the core grant concept in funding cancer centers. Individual projects in a cancer center will then compete as traditional or program project grants in program areas. As a fair percentage of chemotherapy research is carried out in cancer centers, this new approach will lead to significant increase in the Chemotherapy Program.

Development of the Program along the lines described above has slowed as a result of the departure of the Program Director to become Director of the Cancer Control Program. However, active recruitment is under way to obtain a successor who will be able to maintain the vigor already present in this new and burgeoning program.

The Program continues to provide scientific direction for grant-supported research involving the use of drugs in the treatment of cancer. The four major thrusts of the Program are: (1) clinical therapeutics; (2) developmental studies; (3) drug toxicity; and (4) cancer-related disorders.

Clinical Therapeutics

Clinical trials involving Phase I (dose/toxicity trial), Phase II (efficacy trial), Phase III (comparative trials), and adjuvant studies continue to be most effectively and efficiently carried out and completed through the protocols of the clinical cooperative group. Seldom are enough appropriate cases available to a single institution for completion of a broad spectrum Phase II study or a prospective randomized comparative Phase III trial. Thus, there are no traditional grants for clinical trials being funded at this time.

In recent years the clinical cooperative protocols have shifted from Phase I and II studies-to the Phase III trials, and from acute leukemia studies to trials in the so-called solid tumors. More than half of the protocols of the clinical cooperative program during 1974 were Phase III studies.

By charge from the Cancer Clinical Investigation Review Committee, initial scientific review of cooperative group protocols rests with CIB staff and has been an important responsibility of the Program Director for Chemotherapy.

About 50 percent of these protocols required significant major protocol revision and 30 percent needed only minor revision; the remaining 20 percent of the newly submitted protocols were processed by the program staff without change. Where indicated, additional review of protocols is sought from other program directors in the CIB, from CCIRC members, and/or ad hoc scientists. Such additional review was requested in 10 percent of the protocols, usually for those studies which involved radiation as a primary or important aspect of the therapeutic plan. Modifications of group protocols may be required while a study is in progress. These modifications are formulated as addenda to the protocol, and again these are carefully reviewed and processed by this Program. This central protocol review resource has proved expeditious, has been well accepted by the scientific community involved in the studies, and has provided the potential for continuity and coordination of the scientific work of these groups.

To foster the coordination of clinical trials among the various clinical cooperative groups, nongroup participating clinical United States scientists, other treatment-oriented segments of the National Cancer Institute, and foreign clinical scientists, including Russian investigators, the Clinical Cooperative Group Protocol List was completed and put on the computer during fiscal year 1974. The listing includes protocols by disease area as well as by the initiating cooperative group, and will be updated and distributed every six months.

In addition to protocol responsibilities and maintenance of close scientific liaison with the groups through attendance at group meetings and regular phone communication, the program assists the CCIRC in its review of the cooperative group, particularly in connection with group site visits. The Program Director participates in such site visits in a staff capacity and later aids the Chief of the CIB in transmission of CCIRC and National Cancer Advisory Board recommendations to the groups. Thus, the Chemotherapy Program serves as a focal point for the clinical trials of the cooperative group program and also provides staff assistance to the CCIRC in its regular review of the components of the entire Program.

Developmental Studies

Support of these basic studies is essential to provide clinical investigators with rational bases for organization of clinical trials involving drugs. As drug action depends on proliferative potential of malignant cells, studies of the kinetics of solid tumors and leukemia are in progress. Particularly well developed are studies of the kinetics of normal human cervical tissue, cervical carcinoma in situ, and invasive cancer. Based on these kinetic observations, treatment programs by both perfusion techniques and systemic administration are under way. As the drug activity may be maximum in certain aspects of the cell cycle, synchronization of malignant cells would seem desirable. Computer science and statistics have techniques available which are being explored in acute leukemia to develop mathematical models which correlate cellular kinetic data and drug action in an attempt to provide productive information for therapeutic programs. Predicting potential

tumor-drug sensitivity at a cellular level and measurement of response, especially in solid tumors where response indications of tumor shrinkage can be quite gross, have been vexing problems. The recent successful development of a field ionization mass spectrometer has the theoretical potential of marked sensitivity and the need for only minute tissue quantities for analysis. Animal tumor system testing with this instrument is now under way. Studies of granulocytopoiesis, its inhibitors and stimulators continue. Analyses of pancreatic secretion to determine difference between malignant and nonmalignant disease and chromosomal analyses of leukemic and preleukemic states under program support may not only be of value as diagnostic tests but also may serve as markers to determine response to treatment.

Recent work has renewed interest in the role of blood coagulation in the metastatic neoplastic process. Traditional grant projects studying coagulation in malignancy are being funded. One cooperative group study is assessing the usefulness of this approach. Pharmacokinetic studies of a number of drugs are under way in a number of cooperative groups.

Drug Toxicity Studies

All drug treatment programs involve toxicity to normal tissue. The usual toxicity event involves the bone marrow but recently unusual toxicities have been recognized from drugs being used in the cooperative group protocol, i.e., the cariomyopathy of Adriamycin and pulmonary toxicity of Bleomycin. These toxicities occur only at total cumulative dose levels for each drug and thus limit course of treatment. In the traditional grants area, a number of studies are exploring the more usual types of bone marrow toxicity. White blood cell and bone marrow preservation with newer agents and with lysosomal enzyme stabilizers are under way. The development of an animal model for study of platelet transfusion resistance has been used in early studies of the means of preventing such resistance. Since drug doses are often modified due to decreases in the peripheral total white blood cell count, several interesting techniques for assessing granulocyte reserve are in progress. These have the potential advantage of greater simplicity and less side effects than the more "standard" tests, etiocholanolone or endotoxin tests. Studies on white cell defects which lead to increased susceptibility to infection suggest that intracellular sequestration of organisms in phagocytic white blood cells may lead to protection of offending microorganisms from antibiotic treatment. Areas still to be explored and of vast interest are those which lead to protection of normal tissues during drug treatment.

IMMUNOLOGY PROGRAM

Fiscal year 1974 constitutes the fifth year of a formal Immunology Program within the Division of Cancer Research Resources and Centers. The following table indicates growth of the program as judged by the money invested and numbers of grants funded.

FY	Total Amount	R01		P01		T01	
		No. of Grants	Total Amount	No. of Grants	Total Amount	No. of Grants	Total Amount
70	$ 5.3	--	--	--	--	--	--
71	$ 5.3	63	$ 3.1	3	$ 1.3	14	$ 1.0
72	$ 7.2	134	$ 4.8	5	$ 1.4	15	$ 1.1
73	$13.0	148	$ 9.0	8	$ 3.0	13	$ 1.0
74	$19.0	214	$15.0	9	$ 3.7	9	$ 0.5

NOTE: Dollars in millions. Figures thru November 1973 Board.

It can be seen from the table that while growth was slow in the early years of the program, momentum has increased in fiscal year 74 to the point where over $19.0 million is invested in this area. Because of the increased interest in tumor immunology, the number of grant applications is increasing rapidly. The rate of approval has also increased, due at least in part to the attraction of more highly qualified immunologists into the area. The financial investment in the grants immunology program -$19.0 million- compares favorably with that in the contract immunology program which at the time of this assessment was near $10.0 million. A staff of three--program director, program analyst, and secretary--backed up by an expert financial management section easily manage this federal investment in grants.

Over the last few years the program director has become increasingly aware of immunology grant assignments being made to other institutes that could logically come also to the Cancer Institute. Within the last year we may have made progress since we are now receiving more dual assignments. Of those assigned during the last year, five were paid on which Cancer was secondary and three were paid which had no original Cancer assignment. This problem of not having a dual assignment penalizes the investigator severely in that he is delayed three to four months while his grant awaits approval by the National Cancer Advisory Board.

The Tumor Immunology Program is concerned in general with the application of immunological concepts and techniques to the solution of problems encountered in the diagnosis, prevention, and treatment of neoplasia. Both animal hosts and man respond to antigens associated with tumors by the production of both humoral and cell-bound antibody. Considerable current research within the program is concentrated in the area of study of the types and attributes of cells associated with these responses. Lymphocytes concerned in the immune response are predominantly of two types -- B cells

and T cells. B cell research centers around the production of humoral antibodies and T cell research around the cell-mediated immune responses. There is increasing evidence that the macrophage, as well as B and T cell lymphocytes, plays an important role in protection against malignant disease. There is still considerable interest in the roles of humoral antibody as protective antibody and a "blocking" or "enhancing" antibody, in which case it reacts with the antigen at the malignant cell surface, thereby preventing immune lymphocytes from attacking and destroying tumors cells. These immune responses to tumor antigens, once fully understood, can be exploited for purposes of diagnosis, therapy, and probably prevention of neoplastic disease.

Although no unanticipated "breakthroughs" have occurred this year, forward strides have been made in several directions, as follows.

1. Investigators continue to use bacterial adjuvants, especially BCG, in the treatment of various malignancies. As more investigators enter the field, thus increasing the patient load, and as more controlled studies are done, it should be possible to determine whether this approach to therapy is worthy of continued pursuit. There is evidence at this time that non-specific resistance is increased but there are also reports of untoward reactions to the use of BCG. Other organisms, including Corynebacterium parvum, and Listeria monocytogenes have also been used to induce non-specific immunologic responses. Possibly more work of this nature is currently being supported by contract than by grant, whereas grant-supported research in the area is directed more at understanding the mechanism and refining the technique of the use of non-specific adjuvants.

2. A vast amount of work is being done on the effect of mediators of the immune response in producing specific immunity to tumors. Work is progressing nicely on the use of immune RNA to transfer tumor immunity in experimental animals and in the use of transfer factor in the treatment of human disease. Immune RNA has been successfully adapted to heterologous and syngeneic systems of experimental animals but has not yet been tried in humans. Transfer factor has been used in leukemia and melanoma without yielding very promising results. Much more work is necessary, however, before a definitive judgment can be made on the possible advantages of transfer factor in therapy.

3. As mentioned earlier, many investigators in the program are working on the reactions of T and B lymphocytes and macrophages in tumor immunity of both experimental animals and man. Not only are fundamental problems being investigated, but methods for the determination of reactivity of the various cells are being developed and tested in clinical settings. It is still highly desirable to learn which test or tests for cell mediated immunity correlate with clinical improvement in the patient.

The success of the Immunology Program and its rapid growth have been due in no small part to interaction with a group of highly motivated advisors to the program. These include: Dr. Bernard Amos, Duke University Medical Center; Dr. Martin Flax, Tufts University School of Medicine; Dr. Ingegerd Hellstrom, University of Washington School of Medicine; Dr. George Santos, Johns Hopkins University School of Medicine; Dr. Stewart Sell, University of California San Diego; Dr. Barry Bloom, Albert Einstein University; and Dr. Henry Winn, Massachusetts General Hospital. These people continue to devote their time and skills to the discussion of problems of research, communications and training as applied to the field of tumor immunology.

During this fiscal year the following accomplishments can be reported:

1. Research - The group has continued its interest in delineating neglected areas of tumor immunology and those in need of greater emphasis, and in making these known to the scientific community. The scientific community is increasingly responding to these program needs as they are made aware of them. Senior immunologists, many of whom were creative leaders in transplantation immunology a few years ago, are rapidly joining the tumor immunology field. This is witnessed by the fact that for the June 1974 Board Meeting there are eight program project grants in immunology submitted from quality investigators. Individuals in distinguished foreign institutions, especially the Chester Beatty Research Institute, London and the Walter and Eliza Hall Institute in Melbourne are increasingly applying to the Cancer Institute for support of tumor immunology projects.

2. Training - From the results of a questionnaire sent out last year, it was possible to document the need for increasing the number of individuals trained in the techniques of immunotherapy. This data was used in the compilation of arguments in support of training and resulted in the inclusion of tumor immunology on the preferred list for fellowship support. A record number of applications in immunology is anticipated.

3. Communications - The program sponsored (and the Program Director was the Chairman of) the first Gordon Conference in tumor immunology held at Holderness School in Plymouth, New Hampshire in July of 1973. The program was concerned primarily with controversial areas in tumor immunology and was attended by 135 individuals, including several from foreign laboratories. It was enthusiastically received by the scientific community and will be repeated in January of 1975 at the Gordon Conference site in Santa Barbara, California. The chairman for the second meeting will be Dr. Bernard Amos and the co-chairman Dr. Ingegerd Hellstrom, both in advisory positions to the grants immunology program. In addition, the program together with the contract immunotherapy program, sponsored a meeting of persons holding either a grant or contract in tumor immunotherapy. This was attended by about 70 people holding grants and 20 holding

contracts. It was held at Jekyll Island, Georgia, in January 1974. The meeting appeared to be worthwhile, with attendees responding enthusiastically. A similar program in an area other than immunotherapy may be held in the future or, as some prefer, another meeting of the nature of the all grantee one, held in Woods Hole in October 1972 may be the selected alternative. In addition to these conferences planned and sponsored by the Immunology Grants Program, a Radiation-Immunology Conference held in April 1974 was funded, and appropriate contributions are being made to both the International Meetings of the Immunology and Transplantation Societies. The Program Director has served as Chairman of sessions in tumor immunology programs presented by the joint sponsorship of ACS and NCI in New York in December of 1973, and at the American Association of Cancer Research Meeting in Houston in March of 1974. In addition she was an invited speaker at a session on research funding held in conjunction with the Symposium on Onco-fetal antigen at Oak Ridge, Tennessee in November 1973.

This grant program, together with the immunology contract program and other immunological research supported by other mechanisms --the American Cancer Society, the National Science Foundation, etc.-- should make a substantial contribution to the National Cancer Plan within a few years time if the funding level is adequate. We are confident of the continued strong support of the scientific community in this grant-supported program, and we look forward to preserving a climate in which creative investigators can pursue problems related to our goals without direction or interference on the part of government managers. We take special note, however, that even without direction, members of the scientific community are eager to participate and communicate with us continually on the progress of their grant-supported research, as well as other relevant research.

DIAGNOSTIC RESEARCH AND PREVENTION PROGRAM

This program is primarily involved in programs and projects related to prevention, early detection, diagnosis, treatment and rehabilitation, and the application of such knowledge to the population at risk and concurrent dissemination of this knowledge to health professions. In these areas the program is concerned both with improvement of currently existing techniques as well as the development of new modalities. To accomplish this the program has three major objectives: (1) develop mechanisms for the selection of high risk populations through the identification of predisposing and commonly associated laboratory and clinical factors and, through computer technology, utilize these factors as criteria for selection. (2) Evaluate and improve known diagnostic procedures by applying them to selected groups. (3) Utilize recent leads in the field of biological science, instrumentation, and computer technology to develop new diagnostic procedures. The program maintains a continuing survey and evaluation of the state of knowledge and the clinical aspects of cancer, making special note of developments and accomplishments in advancing the prevention, detection, diagnosis and treatment of cancer in man. In addition, the program is concerned with identifying special needs and opportunities where increased or specialized manpower facilities and effort might be productive. As new opportunities for advancement in the above areas are identified, the program will be responsible for developing activities in these areas and provide for continuing technical administration of such new and/or special programs once such programs are developed.

There are certain cancer sites which, either because of the magnitude of the problem or because of recent advances in technology, will warrant either new program development or expansion of existing ones. In fiscal year 1974, the sites that were targeted initially included: 1) cancer of the uterus, 2) cancer of the breast, 3) cancer of the head and neck, and 4) cancer of the lung. In order to maximize the funds made available to this program, increased emphasis was given to this program's main goals, concentrating on the above cancer sites, as well as continuing to develop mechanisms for the selection of high risk populations.

In fiscal year 1974, the base budget for the Diagnostic Research and Prevention Program was $990,000 (actual awards) with approved but unfunded projects remaining in the amount of approximately $531,634.

SURGERY PROGRAM

The Surgery Program has remained a small one for a number of reasons. Perhaps the primary one is the lack of a fulltime Program Director. A major factor here is the impressive shortage, nationwide, of academically oriented surgeons whose primary research interests and competence are in cancer. This is a long-standing deficit, only very recently accepted as such by the academic surgical community generally. Programs for the training of academic surgical oncologists are being developed but are not yet productive.

The phenomena noted above are in a sense paradoxical, since cancer is one of the more common disease areas represented on surgical services throughout the country, and surgical treatment for localized cancer has long been the mainstay of successful therapy.

NATIONAL ORGAN SITE PROGRAMS BRANCH

The National Organ Site Cancer Program consists of grant-supported national projects of targeted cancer research. Each national project is a planned and integrated research effort oriented toward cancer of a specific organ site. The planning, direction, coordination, and scientific administration of each national project is conducted at a headquarters institution other than the National Cancer Institute. The National Project Director, who is not an employee of the National Cancer Institute, is assisted in the planning and in the scientific administration of the national project by a Working Cadre of active research scientists from institutions throughout the nation.

The National Cancer Advisory Board (NCAB), after reviewing comprehensive plans for national programs of research, recommended the implementation of three national organ site projects. In March 1972 the Board recommended initiating the National Bladder Cancer Project under the direction of Dr. Gilbert H. Friedell, St. Vincent Hospital, Worcester, Massachusetts; in June 1972, the National Large Bowel Cancer Project under the direction of Dr. Murray M. Copeland, M. D. Anderson Hospital and Tumor Institute, Houston, Texas; and in November 1972, the National Prostatic Cancer Project under the direction of Dr. Gerald P. Murphy, Roswell Park Memorial Institute, Buffalo, New York.

These national projects are now well underway. Currently 44, 38, and 32 active grants support the research projects in the National Bladder, Large Bowel and Prostatic Cancer Projects, respectively. During the year, each of the national projects held a workshop in which grantees discussed their projects and other interested scientists also participated. These workshops were planned to enhance communication and interchange of ideas between investigators with disparate scientific disciplines but with interests in common disease problems.

In November 1973, the Board approved support for developing the necessary scientific background and exploring the feasibility of a National Pancreatic Cancer Project under the direction of Dr. Isidore Cohn, Jr., Louisiana State University School of Medicine, New Orleans. In March 1974, the Board approved support for developing the state-of-the-art and research opportunities in head and neck cancer under the direction of Dr. Joseph H. Ogura, Washington University School of Medicine, St. Louis, Missouri.

Two committees, advisory to the NCI and the NCAB, provide a continuing surveillance and evaluation of the overall activities of existing national projects. These committees are: (1) the Bladder-Prostate Cancer Advisory Committee under the chairmanship of Dr. Rubin H. Flocks, University of Iowa; and (2) the Colon-Rectum Cancer Advisory Committee under the chairmanship of Dr. Joseph F. Ross, University of California, Los Angeles.

National Bladder Cancer Project

The National Bladder Cancer Project's planned program of research encompasses the general areas of prevention, detection, diagnosis, prognosis and treatment.

The research ultimately directed toward preventing bladder cancer includes endeavors to identify the hazards and populations at risk as well as to determine the mechanisms of bladder carcinogenesis. The National Project supports investigations of the relation of occupation and of artificial sweeteners to bladder cancer. Investigators in the carcinogenesis area are studying the metabolism of environmental factors such as N-hydroxy and nitroso compounds and dietary factors such as indole and tryptophan, as well as the carcinogen from bracken fern that may enter cows milk. A rapid urine test for bladder carcinogens is being developed, using mouse skin and mutation of bacteria as test systems, and a study of rats fed FANFT is exploring the possible initiation and promotion basis of bladder cancer. Several projects include an interest in the possible role of viruses in bladder cancer.

Interrelated research on detection, diagnosis, prognosis and treatment has high priority in the National Bladder Cancer Project. Several investigations are devoted to improving diagnosis and predicting the clinical course of the disease. These projects include light and electron microscopy of epithelial changes associated with bladder cancer and studies of chromosome patterns of human bladder tumors. Other investigations attempt to automate techniques for detection and diagnosis using urinary cytology and tumor tissues. Several investigators are attempting to identify biological, biochemical and/or immunological means of detecting or classifying bladder tumors and patients.

A clinical study involving seven participating clinical institutions, a statistical center, and a central cytology and pathology laboratory got underway during the year. This study is concerned with alternative clinical pathways that individuals with bladder cancer may follow as well as the vesical epithelium in areas considered non-cancerous by cystoscopic observation in patients with bladder cancer and in patients with positive urinary cytology. Two treatment protocols include a study of intravesical installation of Thiothepha on non-invasive bladder cancer and a study of external irradiation and combined irradiation and surgery as definitive therapy for patients with invasive primary bladder carcinoma. Both clinical and laboratory investigations of immunological responses to current therapy, including chemotherapy, may pave the way for further treatment investigations.

National Large Bowel Cancer Project

The National Large Bowel Cancer Project has implemented research in the areas of diagnosis and prevention, immunology and immunotherapy, exper-- imental chemotherapy, cell kinetics and molecular control, and carcino-genesis and epidemiology. Diagnostic investigations include studies of families genetically predisposed to colon cancer and patients drawn from general populations. Conventional diagnostic procedures such as sigmoidoscopy, barium enemas with air contrast and colonoscopy are compared with new techniques such as measurements of CEA and evaluations based on colonic washings through the sigmoidoscope and/or colonoscope, for cytologic enzymatic and immunologic observations.

Modes of inheritance, linkage with marker traits, chromosome analyses and a study of tissue culture characteristics are also being pursued in regard to colon cancer in populations with familial disease. Laboratory and clinical studies are underway to improve the detection of CEA, to investigate other tumor specific antigens and to evaluate these antigens for diagnosing colon cancer. Other studies deal with the immune status of colon cancer patients before and during therapy to evaluate immune tests as measures of effective therapy. Immunotherapy adjuvant to surgery in colorectal cancer patients is also being explored. Studies on cell kinetics and molecular control focus on the intestinal mucosa. These studies include an attempt to synchronize the divisions in crypt cells of the intestinal epithelium. Other investigations are concerned with the isolation, purification and characterization of membrane and secretory glycoproteins from normal and neoplastic colonic mucosa. Still other investigations deal with the differences in enzymes and enzyme activity in normal, premalignant, and malignant colon mucosa.

In the area of carcinogenesis, a number of studies are underway to investigate the mechanisms of known carcinogens in laboratory animals such as nitrosamines and nitrosamides, methoazoxymethanol acetate, 3-methyl-4-aminobiphenyl and the metabolites of bile acids. Carcinogenic effects of asbestos fed to rats are also being explored. Several investigations are concerned with the effects of sex hormones on chemical carcinogenesis. The role of intestinal bacteria on carcinogenesis is also under investigation. Etiological factors in colon cancer are being explored in an epidemiological study by comparing Puerto Ricans in Puerto Rico and New York City.

National Prostatic Cancer Project

The National Prostatic Cancer Project, the most recent of the three national projects to get underway, has made considerable progress in implementing research that encompasses the general areas of treatment, diagnosis and detection, and epidemiology and prevention. A treatment study has begun in five participating institutions to evaluate non-hormonal chemotherapeutic agents in patients with advanced prostatic cancer who fail to respond or no longer respond to existing hormonal therapy. The study is currently investigating 5-fluorouracil and cyclophosphamide in a crossover protocol.

An irradiation therapy investigation is underway to test the hypothesis that once the primary neoplasm extends beyond the prostatic capsule, regional lymph nodes may be involved. Radiotherapy is extended to include the next most proximal uninvolved lymph node group.

Investigations which attempt to develop laboratory techniques for identifying potential prostatic cancer chemotherapeutic agents include a study of the effect of various agents on 5-alpha reductase and arginase activity. Another study measures the effect of drugs on the rate of prostatic DNA synthesis in laboratory animals. In other projects investigators are attempting to synthesize cytoxic agents specific for prostate tissues and prostatic tumors.

In the area of detection and diagnosis, both prospective and retrospective studies are underway to evaluate various staging and histological grading

classifications for estimating survival of patients with prostatic cancer. Still other studies explore laboratory tests of endocrine dependence in explants from prostatic carcinoma, CEA and other possible tumor specific antigens and various immune responses to determine if additional tests can be developed to help in detection, diagnosis and in evaluating the results of therapy.

In the general area of etiology and prevention, several studies are concerned with the possible involvements of viruses in prostatic cancer. These studies utilize both light and electron microscopy as well as biochemical and immunological techniques.

The National Project established a prostate cancer tissue collection center and supports several investigators who are attempting to develop cell and tissue culture techniques for prostatic tumors. An epidemiological study is underway to compare carcinoma of the prostate in a high risk (American Blacks) and a low risk (African Blacks) population group. This study seeks epidemiological factors and compares clinical features such as tumor morphology, incidence of non-invasive carcinoma in postmortem material and the relation between incidential carcinoma and non-neoplastic prostatic changes and changes in liver adrenal glands, breast and testes.

OFFICE OF THE ASSOCIATE DIRECTOR
CANCER CENTERS

The Office of the Associate Director for Cancer Centers, one of three Offices reporting to the Director of the Division of Cancer Research Resources and Centers, directs and coordinates the operations of the Cancer Centers Branch, the Research Facilities Construction Branch, and the Cancer Clinical Education Programs. The Associate Director has responsibility for supervising the administrative, fiscal, reporting and developmental activities of these branches and programs. The activities of the Cancer Centers Branch and the Research Facilities Construction Branch are described below. The third segment, the Cancer Clinical Education Programs, includes the Cancer Clinical Training Grants Program, which is described elsewhere but was largely inactive during fiscal year 1974, as well as the Cancer Clinical Education Grants Program, which is expected to be activated in 1975.

The Associate Director's Office was established in response to the rapidly increasing size of the Centers and Construction programs. In addition, there was recognition of the importance of the Centers as a vital resource in the overall mission of the National Cancer Institute under the National Cancer Act of 1971, which called for the establishment of additional Centers to provide a national network of cancer research and demonstration units.

CANCER CENTERS BRANCH

In response to the need for new knowledge through scientific research and rapid translation of the findings into coordinated care for cancer patients, the National Cancer Institute activated in the early 1960's a Cancer Research Centers Program to provide grants for the support and development of cancer complexes which could engage in clinical and basic research, improve diagnosis and treatment of patients, train an effective cancer cadre for the future, and hasten the upgrading of cancer care in surrounding areas. Cancer Centers have increased in number during the past decade and have been diversified in function according to special facilities and personnel available. There has been increasing emphasis in recent years upon Centers of broad scope which can provide a comprehensive, multidisciplinary attack upon cancer problems. The National Cancer Act of 1971 has given further stimulus to this trend by providing for the establishment of additional Cancer Centers. Cancer Centers will be a vital element in the intensified development and implementation of a nationwide cancer program which is presently underway.

In 1972 extensive efforts were made to integrate data derived from a variety of sources and staff studies, including advice from the National Cancer Advisory Board. As a result, guidelines were prepared for use by institutions that wish to play a role in the National Cancer Centers Program. These guidelines outline the procedures to be followed in the establishment of cancer centers and were published in 1973 in the format of a brochure which has been made available to all interested parties. Further, improved methods of review and evaluation have been developed to insure that the Centers Program will play its essential role within the National Cancer Plan. The guidelines distinguish between two categories of cancer centers: Comprehensive and Specialized. Comprehensive Centers are those conducting long-term multidisciplinary programs in the following areas: cancer biomedical research; cancer clinical services and investigation; cancer training and demonstration and community-oriented programs of cancer detection, diagnosis, education, epidemiology, rehabilitation, and data development and exchange. A Comprehensive Center may have several center grants within it. For example, M.D. Anderson in Houston, Texas, is a Comprehensive Center with four ongoing center grants. Specialized Cancer Centers have programs in one or more but not all of the above areas, in which the research effort, specialized study, or form of patient treatment has resulted in well defined areas of emphasis. It is the intent of the Cancer Centers Program to further strengthen and develop specialized centers.

The guidelines for both types of centers and the methods of review have been extensively modified in 1974 based on experience gained in this rapidly expanding program. Renewed emphasis is being placed on the Cancer Center Support Grant as a "core" grant mechanism to support the administration, key personnel, common services and collaborative activities of cancer centers and to provide "seed money" for development of new programs within the centers. The Cancer Center Support Grant is related to the needs associated with other cancer activities at the Center, which are supported through traditional grant and contract mechanisms. The large "umbrella" grants supporting multiple research efforts are being deemphasized and will

eventually be phased out, with individual research projects or program projects being encouraged to seek separate support in order to facilitate and improve the quality of the review process itself as well as to improve fiscal accountability.

Importance has been placed on an integrated administrative structure for cancer centers in order to encourage applicant institutions to adopt methods demonstrated to be most effective in existing cancer centers and to insure a collaborative effort between the separate component grant activities and the Cancer Center Support Grant activities.

Preliminary population studies have been carried out to determine the optimal geographic distribution of cancer centers based on patient referral patterns so as to make a maximum impact on patient care across the country. The preliminary data indicate that the cancer centers considered to be comprehensive prior to the National Cancer Act of 1971 served approximately 29 million people. By the end of the fiscal year 1974, approximately one-third of the population should be close enough (60 miles) for a cancer patient to visit a Comprehensive Cancer Center for a consultation and return to his home the same day by surface transportation; and the bulk of the total population should have ready access to either a Comprehensive or a Specialized Cancer Center in their geographic area.

Since the guidelines for cancer centers emphasize community involvement, a growing number of localities have expressed interest to the Centers staff in initiating cancer programs. These Community Centers, affiliated with already established cancer centers, will be able to furnish excellent cancer care to patients in communities of modest size which do not have a medical school, bringing to cancer patients, physicians, and paramedical personnel the benefits of the most recent advances in cancer research and treatment modalities. They will receive NCI support for cancer detection, education, research and the development and exchange of information, but will also develop local sources of support for their programs and utilize normal sources of payment for patient care. One Community Center is already funded and underway at the Mountain States Tumor Institute in Boise, Idaho. Two institutions--the Wilmington Medical Center in Delaware and the Luzerne County Medical Society in Wilkes-Barre, Pennsylvania--are planning for such Centers with NCI grant funds. Applications for support have also been received from the Kern Radiation Oncology Center in Bakersfield, California and from the Greenville Hospital System in South Carolina. The number of applications is expected to increase during fiscal year 1975.

In addition, in conjunction with the Cancer Control Program, a new program of Cancer Control Grants in Comprehensive Centers was developed, applications reviewed, and awards funded to provide support for such activities in ten Comprehensive Centers. A contract to the Institute for Scientific Information has been continued to determine the impact of the research performed in cancer centers on the scientific literature. This will facilitate the evaluation of cancer center activities both individually and as a total program. Another contract with a consultant firm aids the

Centers staff in planning and evaluating the effectiveness of recent developments in the Cancer Centers Program.

A program recently initiated in conjunction with the NCI Office of Cancer Communications will utilize the contract mechanism to encourage in each Comprehensive Cancer Center an Office of Cancer Public Information. The primary responsibilities of this Office will be to catalog cancer services for the Center's geographic outreach area, to become the focal point for all public inquiries regarding cancer care and education, to develop educational materials (brochures, films, radio and TV spots) for laymen, and to document and evaluate the impact of these programs with the aid of a consultant firm utilized by the NCI for this purpose. This program will be another mechanism by which the centers will be able to more successfully reach the public, while representing an effective data base for the NCI to better evaluate the impact of its centers programs on the total population.

The National Cancer Institute, through the Centers Program, supported 124 Cancer Research Center Grants in fiscal year 1974 located in 66 institutions for approximately $88 million. Included in the above are 28 new cancer center grants which add 14 additional institutions, thus improving the geographic distribution of the cancer centers throughout the United States. The Centers staff administered 76 continuation grants while carrying out the activities necessary to review 114 grants, 78 of which were new, 24 competing renewals, and 12 supplements. These 114 grants resulted in approvals for 51 new applications, 21 competing renewals and 12 supplements. The amount requested for the original 114 grants was $109 million for direct costs while the amount approved for funding was $36 million.

An important aspect of the Cancer Centers Program is the Exploratory Grant mechanism which allows for an institution to plan for the establishment, development and operation of a cancer center. It is important that the planning effort carefully examine and analyze the various alternatives which exist for the institution to develop a program consistent with the local needs and organizational structure as well as with the National Cancer Plan.

In fiscal year 1974, the Centers Program awarded 32 Exploratory Grants at the estimated cost of $2.7 million; these figures include 18 continuation, six new, and eight renewal grants. Thirty Exploratory Grants requesting $3.2 million were reviewed resulting in 27 approved applications totaling $2.0 million.

In order to provide more uniform cancer resources to communities throughout the United States, new cancer centers have been encouraged by NCI staff in the West, Midwest, Mountain States, and the South. The Fred Hutchinson Cancer Research Center, a Comprehensive Cancer Center under development in Seattle to serve the Pacific Northwest, is the culmination of several years of planning (partially supported by NCI grant funds). This center is an independent, nonprofit corporation governed by a Board of Trustees consisting of both lay and professional members. Representatives are included from the Pacific Northwest Research Foundation, the University of Washington, the University of Oregon, American Cancer Society, Swedish

Hospital Medical Center, Children's Orthopedic Medical Center, Virginia Mason Medical Center, and the States of Alaska, Idaho and Oregon. An extramural program to relate intramural activities of the medical community and to provide interaction with practicing physicians to improve the quality of cancer care will be housed in the Center. In addition to basic and clinical research there will be programs in epidemiology-biostatistics, library services and information, education, both professional and paramedical, rehabilitation medicine, cancer detection, and a cooperative clinical study group (surgeons, radiotherapists, medical oncologists, and pathologists) from Washington, Alaska and Idaho.

Comprehensive Center Programs already existing at the University of Wisconsin in Madison and at Yale University in New Haven have been strengthened markedly in fiscal year 1974 through the creation of strong oncology organizations within the university structures to integrate basic and clinical cancer research and treatment through affiliations with the McArdle Laboratory in Wisconsin, the University of Connecticut in Connecticut, and a number of hospitals in their respective areas to develop effective outreach networks in surrounding communities.

Over a period of several years, the University of Southern California School of Medicine and the Los Angeles County Department of Hospitals jointly developed plans for a Comprehensive Cancer Center with NCI Exploratory Grant support. The establishment of the LAC-USC Comprehensive Cancer Center in 1973 enhanced the development of new and expanded programs of cancer research and coordinated existing programs of the University and several affiliated hospitals. The Center is organized into multidisciplinary, interdepartmental programs involving the Schools of Medicine, Dentistry, and Pharmacy, and hospitals and institutions throughout the country. Additional grant applications have been submitted for the development of a regional outreach cancer control network which will accomplish the interaction of community hospitals, physicians, and other universities with the programs of the LAC-USC Comprehensive Center. In early fiscal year 1974 NCI recognized an additional Comprehensive Center being developed at the Mayo Foundation Medical School under the direction of Dr. Jesse Steinfeld. This Center will serve a surrounding population in the Midwest of approximately 1.5 million people. Major multidisciplinary cancer programs are underway in basic science, chemotherapy of solid tumors and of hematologic cancer, and pediatric oncology. Activities are being rapidly expanded in cancer detection, education and community service, and radiation therapy.

Another Comprehensive Center program is now being developed in Chicago under the leadership of the University of Chicago and Northwestern University. This program was formed through the cooperation of eight Illinois medical schools and several non-academic organizations influential in health care throughout the State as well as in Chicago. Representatives of these organizations have formed a nonprofit corporation, the Illinois Cancer Council (ICC). The individual organizations in cooperation with the ICC will promote cancer diagnosis, research, and education in the state of Illinois. A program of research and care underway at the University of Chicago Cancer Center emphasizes oncological virology, tumor immunology, cell biology, carcinogenesis, and clinical research, and

includes an outstanding lymphoma program. Northwestern University's grant proposal for a cancer program with special interest in the development of medical and gynecological oncology and cancer rehabilitation was funded late in fiscal year 1974. Active research programs are underway in virology, radiotherapy and chemotherapy at Rush-Presbyterian-St. Luke's Medical Center, and cancer programs are being planned at the Chicago Medical School.

A Comprehensive Cancer Center Program is also being developed in the Denver area through the collaboration of the University of Colorado, Colorado State, Children's Hospital, and a large number of other hospitals and health care organizations in the area. These groups have affiliated themselves with the Colorado Regional Cancer Center, Inc., a nonprofit corporation established to develop cancer outreach programs and coordinate them with existing cancer research and treatment facilities. Planning for this Center has been supported by an NCI grant. Major cancer programs are in progress at the Pediatric Oncology Center of the Children's Hospital of Denver and at the University of Colorado (both Denver and Boulder campuses) in a multidisciplinary program of cancer research. Basic research work in cancer is also being conducted at the University's Medical Center. A number of cancer research projects are underway or proposed at the University of Colorado at Boulder and at Colorado Springs, at the American Medical Center at Denver, at Denver General Hospital, at the National Jewish Hospital at Denver, and at Colorado State University in Fort Collins, Colorado.

There are certain problems in the Cancer Centers Program that must be dealt with on a continuing basis in order to achieve its goals. Centers staff have given special attention to more effective utilization of the resources between multiple schools in the same geographic area; to effective coordination of center activities in schools with independent departmental structures; to the review of complex proposals; to implementation of effective outreach programs in communities with independent professional traditions; and to maintaining adequate funding levels for the Centers Program.

Clearly, the Cancer Centers Program in the time following the passage of the National Cancer Act has been characterized by rapid change. Both the public and legislators have recognized the central role of this program in the National Cancer Plan. Fulfullment of its expectations will require a great deal of effort in the future as in the past.

RESEARCH FACILITIES CONSTRUCTION BRANCH

The Research Facilities Construction Branch was initiated as the Research Facilities Construction Program in late September 1971 with a public announcement of the availability of limited grant funds to create new cancer research facilities. The essence of this announcement is set forth in the following paragraph:

> "In accordance with the President's call for an expanded, intensified, and coordinated cancer research program, and under authority provided by the Congress in the fiscal year 1972 appropriation act, the National Cancer Institute is initiating a program of grant supported construction of cancer research facilities. The intent of this program is to create new physical resources for cancer research through Federal participation in the cost of new construction and of renovation. Support may be provided for the construction of facilities such as basic research laboratories; clinical research facilities; animal facilities; and associated core, administrative, laboratory, and service space. In all instances, the facilities proposed must be intended for expansion of cancer research and must be used for cancer research for at least 20 years. A purpose of this program, in addition to strengthening research capabilities at existing cancer centers, is to develop new strong multidisciplinary cancer efforts in regions of the country where they do not exist. Thus, in the consideration of applications some attention will be given to geographical distribution as well as to the relation of proposed construction to existing centers of excellent cancer research."

Justification of construction funds has been, and will continue to be, in terms of new cancer research programs. The primary review and evaluation of applications has been essentially scientific, considering such matters as scientific merit of the proposed program(s), the technical competence of the applicant institution staff, the intellectual environment of the applicant institution, and the scientific and fiscal administrative capabilities of the applicant institution. In addition, other criteria have been considered, such as the geographical location of the applicant institution, the applicant institution performing an essential role in the National Cancer Program, the promptness with which construction can be gotten underway, space request commensurate with projected program scope, essential minimal facilities for those institutions with an undeveloped cancer research program, net space utilization of 60 percent or more, reasonable cost per gross square foot, acceptable design criteria, etc.

During the first three quarters of fiscal year 1974, five new construction grant applications were reviewed and presented to the National Cancer Advisory Board. Two of these five applications are approved for funding while two were disapproved and one was deferred for future review. Those institutions which were awarded construction funds through the third quarter were the University of Chicago (1 C06 CA14696-01), Memorial Hospital for Cancer and Allied Diseases (1 C06 CA14628-01), Sloan-Kettering Institute for

Cancer Research (1 C06 CA15081-01), Children's Hospital in Cincinnati (1 C06 CA15014-01), University of Washington (1 C06 CA15024-01) and a partial award to the University of Southern California (1 C06 CA14800-01).

During the fourth quarter of fiscal year 1974 it is anticipated that construction grant awards will be made to the University of Wisconsin (1 C06 CA15002-01), Yale University (1 C06 CA16360-01) and the balance of the award to the University of Southern California. These three remaining awards will total approximately $15.6 million.

With the awarding of the above grants, there will remain an approved but unfunded backlog amounting to over $17.3 million. Included in this group are the Salk Institute for Biological Studies (1 C06 CA13706-01), Hahnemann Medical College and Hospital (1 C06 CA14202-01), Tufts University (1 C06 CA14206-01), Howard University (1 C06 CA14911-01), Albert Einstein College of Medicine (1 C06 CA15023-01) and Wasington University (1 C06 CA16082-01).

Through the third quarter of fiscal year 1974 there are 16 of 41 projects currently under construction contract at various stages of completion in this program. These institutions include Yale University, Children's Cancer Research Foundation, M.D. Anderson Hospital and Tumor Institute, New York University Medical School, the Ohio State University, the Worcester Foundation, Roswell Park Memorial Institute, Bowman Gray School of Medicine, UCLA, Rockefeller University, the University of New Mexico, St. Jude Children's Research Hospital, the Trudeau Institute, Massachusetts Institute of Technology, Fred Hutchinson Cancer Research Center and the University of Wisconsin. All totalled, by the completion of fiscal year 1974, over $108 million will have been awarded under the cancer construction grant program. Of this total amount approximately $70 million have been devoted to the Comprehensive Centers and $38 million to Specialized Centers.

Adequate staff was initially assigned to review the grant load, but with the increasing number of award projects moving toward the active construction phase, a shortage in general engineering capabilities became evident. In addition, the program must now respond to the newly formulated requirements of the National Environmental Policy Act. This program was partially relieved during the third quarter by the hiring of an additional engineer uniquely qualified to deal with the general engineering and specialized environmental problems.

Program projections into the fiscal year 1975 are difficult, to say the least. It is anticipated that the available funds will range between $17 and $25 million for grant supported projects. In addition to the aforementioned approved but unfunded backlog, the National Cancer Advisory Board will have before it at the June meeting over $43 million in new applications from nine institutions. Further, there are at least five new applications requesting over $18 million in support of new construction pending for the November Board meeting. It is clear that even with a low approval rate the fundable approved applications will exceed the available funds by a considerable margin.

TRAINING PROGRAMS
RESEARCH FELLOWSHIPS PROGRAM

The regular Postdoctoral and Special Research Fellowships of the National Institutes of Health were phased-out on January 31, 1973.

Although no new awards were issued and no new applications accepted after that date, future year commitments for Fellows on duty were honored. The National Cancer Institute had 93 Fellows on duty, 26 of these had one or more years of future support. The total expenditure for the program in fiscal year 1973, was $990,538, representing the six months prior to the phase-out.

Since the training programs were terminated, without prior notice, during the regular winter meetings of the review committees, there were an undetermined number of applications both reviewed and unreviewed in-house at the time. The National Cancer Institute was holding approximately 118 approved new and renewal applications for about $950,000. In addition, an unspecified number of accepted but unreviewed applications were being held in the Division of Research Grants, National Institutes of Health.

With the announcement of the new Individual Postdoctoral Research Fellowship on November 16, 1973, and the release of the impounded funds shortly thereafter, applicants whose requests had been approved were offered funding from either program. Unreviewed applications were scheduled for review, if applicants so desired.

The response was immediate. The National Cancer Institutes received approximately 561 applications for the February 1 deadline which are currently being reviewed. Four of these have requested consideration under the old program. The number of applications formerly received for each deadline averaged between 95 and 100, making the response to the February deadline almost six-fold. Approximately $5,450,000 will be available for funding these applications.

The Individual Postdoctoral Research Fellowship offers research training support in this country and abroad to American citizens, or those lawfully admitted to the country for permanent residence, who have received a doctoral degree in the biomedical sciences.

Policies governing this program stipulate that the maximum training support offered is three years. Individuals who have received three years of National Institutes of Health postdoctoral training support are not eligible to apply.

Furthermore, applicants must certify in advance that they will reimburse the Government the amount of their fellowship stipend if they do not remain in the research and/or teaching careers upon completion of fellowship training.

Fellowship stipends begin at $10,000 a year with annual increments of $400 to a maximum of $14,000. A sponsor's supply allowance of $3,000 for each

fellow is awarded to the host institution, to be expended at the discretion of the Sponsor on behalf of the Fellow for tuition, consumable supplies, meeting travel, etc.

The National Cancer Institute offers fellowship support for research training in the following areas: Cancer Biology, Carcinogenesis, Chemotherapy, Epidemiology, Immunology, Drug Development, and Radiation.

RESEARCH CAREER DEVELOPMENT PROGRAM

The purpose of the Research Career Development Award is to foster, by providing salary support, the development of young scientists with outstanding research potential for careers of independent research in the sciences related to the cancer problem.

The Career Award Program and the Career Development Award Program were both initiated in 1961. In 1964, the Career Award Program, providing support until awardee's retirement, was discontinued. On January 31, 1973, the Career Development Program was phased-out.

At the time of the phase-out, the National Cancer Institute had 63 active awardees for $1,446,562. The combined future year commitments for these awardees was $4,643,935 through fiscal year 1977. A total of 15 applications for $320,829 had been approved but were unfunded because of the phase-out. There were also 39 unreviewed applications which had been received and were being held in the Division of Research Grants of the National Institutes of Health.

With the release of the impounded funds, the Career Development Award Program was reinstated. Twelve of the previously approved applications were awarded for $225,279. The unreviewed applications held by the Division of Research Grants were channelled into the review system. It is expected that approximately 10 of those recommended for approval will be funded.

The funding of this program is conservative since each award has a commitment for four additional years of future support.

The total Career Review Development Awards issued thus far in fiscal year 1974 is 32 for $737,451. The total from 1962 to the present is 933 for $21,220,475.

The program has always received more requests than can be awarded. An increased number of requests is anticipated with the reinstatement of the program.

APPENDIX

Published Results of Scientific Work

Supported by Programs of the Division of

Cancer Research Resources and Centers

National Cancer Institute

July 1, 1973 through June 30, 1974

Prepared by

Program Analysis and Evaluation Branch

PUBLISHED RESULTS OF SCIENTIFIC WORK SUPPORTED BY PROGRAMS OF THE DIVISION OF CANCER RESEARCH RESOURCES AND CENTERS

Since 1938 over 16 thousand research grant applications have been submitted to the National Cancer Institute and during that period of time, $1.3 billion have been awarded to grantees to conduct research on all aspects of the cancer problem. The first grant was awarded to Dr. Louis Fieser of Harvard University for the synthesis of organic compounds to be tested for their cancer causing attributes. Thirty-six years later, the area of chemical carcinogenesis is still actively being persued, as are all aspects of cancer research, ranging from the most basic studies of nucleic acids to the clinically sophisticated investigations utilizing new high-linear-energy-transfer radiation for the treatment of cancer patients.

As results of experiments become known, every grantee is urged to publish the data in medical journals. This report, prepared by the Program Analysis and Evaluation Branch, attempts to review the data published by the grantees during the year. The source of individual NCI support for each paper cited appears as a notation (identification number) with each reference; symbols of the appropriate program areas are also included.

CHEMICAL CARCINOGENESIS

Aryl hydrocarbon hydroxylase (AHH) is a microsomal mixed function oxidase involved in the activation of many carcinogenic polycyclic aromatic hydrocarbons. Induction of this enzyme by benz[a]anthracene was studied in mouse 3T3 cells.[1] Enzyme induction was markedly greater in cells grown in the presence than in the absence of serum and was higher in nonconfluent than in confluent cells. Moreover, addition of dibutyryl cyclic guanosine 3',5'-monophosphate (a putative signal for increased cell proliferation) markedly potentiated induction of AHH. These results suggest that the inducibility of the hydroxylase system by benz[a]anthracene is greater in proliferating than in non-proliferating cells.

Evidence for a possible functional relationship between AHH and reduced-NAD: lipamide oxidoreductase (DT diaphorase) has recently been reported.[2] Treatment of rats with 3-methylcholanthrene, an inducer of AHH, caused a several-fold increase in hepatic DT diaphorase activity. An AHH inhibitor, 7,8-benzoflavone, was an equally potent inhibitor of DT diaphorase in this study. It is suggested[2] that either DT diaphorase might act as a cytochrome P-448 reductase component of the AHH system, in a way analogous to the generally assumed role of NADPH-cytochrome C reductase in cytochrome P-450-linked hydroxylations; or, alternatively, DT diaphorase may be involved in the induction of the AHH system caused by 3-methylcholanthrene, perhaps by providing a component necessary for its biosynthesis.

Hydrocarbons such as 3-methylcholanthrene and certain drugs such as phenobarbital both induce microsomal AHH activity but induction by polycyclic hydrocarbons differs markedly in several major respects from that brought about by phenobarbital. In addition it has recently been shown that although the

specific activity of AHH associated with the nuclear envelope from rat hepatocytes is increased 17-fold by 3-methylcholanthrene, it was not affected by phenobarbital.[3] Under the same conditions, the microsomal hydroxylase level was increased 8.5-fold by 3-methylcholanthrene. Thus, although the components of the TPNH electron transport chain are integral parts of both the nuclear and microsomal membrane systems, biochemical regulation of nuclear membrane AHH differs from that observed for the microsomal enzyme.

Most studies of carcinogen metabolism utilize hepatic enzymes since the liver is the major site of metabolism of systemically administered carcinogens. However, 7,12-dimethylbenz[a]anthracene (DMBA) has been demonstrated to induce cancer in mammary explants in organ culture.[4] This clearly suggests that metabolic activation of the carcinogen can occur in the mammary gland itself. Enzymes capable of converting DMBA to hydroxymethyl derivatives have now been demonstrated in rat mammary tissue.[5] Several differences in DMBA metabolism were noted in the mammary tissue as compared with liver. Among these was that whereas pretreatment with 3-methylcholanthrene shifts metabolism from side chain to ring hydroxylation in the liver, the mammary gland retains its ability to terminate the hydroxylation of DMBA at the monohydroxy stage. This ability to form the 7-hydroxy derivative by the mammary gland is markedly decreased as a result of 3-methylcholanthrene pretreatment.

Because of the suggestion that some carcinogens function by interacting with hormone receptors in a way that alters their normal activity in a cell, binding of ^{3}H-3-methylcholanthrene (3-MC) to macromolecular components of the rat uterine cytosol fraction was analyzed and compared with the specific binding of estradiol to its receptor.[6] The 3-MC bound to a component which sedimented as a 5S complex and was easily distinguished from the 8S estradiol-receptor complex. This result and associated findings negate the possibility that the carcinogen acts in estrogenic target tissue by directly interfering with the normal interaction of estrogens with their receptor sites.

The in vitro binding kinetics and specificity of 20-MC interaction with mouse epidermal cytosol has been investigated in the presence and in the absence of microsomal metabolizing systems.[7] A major portion of the binding appeared in a single electrophoretic peak with the same mobility as mouse serum albumin, but believed not to be identical with this substance. In the presence of microsomes plus NADPH, the binding was partially covalent. Incubation of mouse serum albumin with labeled 20-MC in the presence of competing non-radioactive carcinogens or promoters demonstrated little or no inhibition of binding. In vitro competition by other hydrocarbons or by promoters for binding of 20-MC to the cytosol receptor protein showed an impressive correlation with carcinogenic and promoting activities.

No rationale for carcinogenic activity from theoretical calculations of chemical or physical interactions of hydrocarbons with target molecules has yet produced a satisfactory correlation of such interactions with tumorigenic activity.[8] However, many apparent exceptions to the correlations drawn have merely been inadequately tested. Three hydrocarbons (phenanthrene, picene,

and benzo[b]chrysene) previously reported to be nontumorigenic initiated tumor formation in mouse skin in recent tests.[8] The first two of these compounds were expected to be tumor initiators on the basis of previously published theory.

N-hydroxylation is an initial activation step in carcinogenesis by AAF and several other aromatic amines and amides, whereas ring-hydroxylation of AAF is an inactivation process. Pretreatment of adult male rats with the herbicide 3-amino-1,2,4-triazole (aminotriazole) increased the urinary excretion of both N-hydroxy and ring-hydroxy metabolites of AAF.[9] Aminotriazole apparently had a slight inhibitory effect on esterification of N-hydroxy-AAF, a further activation step.

The relative capacity for enzymatic N-acetylation of aromatic amines by liver cytosol has been investigated in several mammalian species displaying different organ susceptibilites.[10] The results indicate that while N-acetylation may be required for hepatic aromatic amine carcinogenesis it is probably not involved in carcinogenesis of the urinary bladder. This supports the suggestion[11]that one of the proximate carcinogens involved in urinary bladder carcinogenesis may be an N-hydroxyarylamine. Acceptance of the involvement of the hydroxylamine metabolite in bladder carcinogenesis has been hindered by the fact that aryl hydroxylamines are water-insoluble and, unlike arylhydroxamic acids, are highly unstable in aqueous media.[12] Evidence has now been obtained[12] for the presence of substantial quantities of a glucuronic acid conjugate of N-hydroxy-4-aminobiphenyl in the urine of dogs given 4-aminobiphenyl. The conjugate is very acid labile, readily liberating free N-hydroxy-4-aminobiphenyl at lowered pH and in the presence of β-glucuronidase.

Another organ susceptible to aromatic amine-induced tumors is the mammary gland. Systemic administration of AAF to female Sprague-Dawley rats causes mammary tumor formation but it is not known whether the same metabolites are involved as those which cause liver tumors in this species. The compound is N-hydroxylated by rat liver and the hydroxamic acid is then converted to an unstable ester. Topical application of N-hydroxy-AAF to female rat mammary tissue resulted in a 70 percent tumor incidence at the site.[13] The tumors, which were mammary adenocarcinomas, appeared 3 to 6 months after the application. In contrast, the tumor incidence after topical application of AAF was 30 percent, the latent period was 8 to 11 months, and the tumors were not confined to the site of application. Additional experiments, comparing the microsomal N-hydroxylation of N-2- and N-3-AAF by mammary gland and liver, showed that mammary gland microsomes, in contrast to those of liver, did not N-hydroxylate the arylamides.[12] There was no evidence that the mammary gland formed the N-sulfates of the N-hydroxy metabolites. The data suggest that N-hydroxylation may play a role in mammary carcinogenesis but that the two-step activation mechanism believed to initiate hepatocarcinogenesis by AAF is not operative in the mammary gland.

Most chemical carcinogens which have been examined lead to some type of covalent substitution of bases in DNA.[14] The reactions of carcinogen metabolites with DNA and the factors affecting such reactions have been the subject of several recent studies. Binding of 3-methylcholanthrene to DNA of the

preneoplastic nodule outgrowth of mouse mammary gland has been found not to be influenced by or dependent upon the rate of DNA synthesis in the tissue.[15] The carcinogen appears to bind to replicating and non-replicating DNA to the same extent. Thus cells in DNA synthesis are not more susceptible to binding of the carcinogen to the genome. Since cells undergoing DNA synthesis are known, however, to be more susceptible to the tumorigenic action of 3-methylcholanthrene, the possibility exists that binding of the hydrocarbon to replicating DNA may improve the chances of inducing aberrations in the nascent molecules, leading to altered genetic regulation in the progeny cells.[15]

Analysis of the structural and functional properties of oligonucleotides and transfer RNA modified by N-acetoxy-N-2-acetylaminofluorene, an active metabolite of AAF, led to proposal of a specific conformation for the structure of nucleic acids in the region to which the AAF residue is covalently bound.[16] According to this model, the attachment of AAF to the C-8 position of guanosine requires a rotation of the guanine base around the glycosidic bond from anti to syn; in addition, the modified guanine base is displaced from its normal coplanar relation with adjacent bases by the fluorene residue and the latter is stacked coplanar with an adjacent base. Reaction of natural nucleic acids (calf thymus and salmon sperm DNA, reovirus RNA, E. coli ribosomal RNA) with N-acetoxy-AAF has now yielded results consistent with this "base displacement model".[17]

An attempt has been made to characterize the inhibition of rat liver RNA synthesis observed after administration of N-hydroxy-AAF. Results indicate that the inhibition affects predominantly ribosomal RNA of nucleolar origin and that it is due to impairment of the nucleolar DNA template rather than of the RNA polymerase.[18] The nucleolus is the site of guanine-rich ribosomal RNA synthesis (see references in[18].)

There seems little doubt of the importance of the ester-forming and -reacting sequences in AAF and N-hydroxy-AAF hepatocarcinogenesis. N-acetoxy-AAF, as mentioned, reacts efficiently with guanosine in vitro, yielding products identical with those obtained from rat liver after administration of N-hydroxy-AAF. There is now evidence that the reactivity of N-acetoxy-AAF is a special case, atypical of the chemistry of N-acetoxy-N-arylacetamides, and that this special position arises from the rapid transition of the N-2-fluorenyl-N-acetyl-nitrenium ion from a relatively high-energy singlet (nonradical) state to a triplet (radical) ground state.[19] It may in fact be that the less likely an N-acetoxy-N-arylacetamide is to generate a radical cation, the stronger carcinogen it will be, although N-acetoxy-4-acetamidobiphenyl is a clear exception to this hypothesis.[19] Studies with N-acetoxy-acetamidophenanthrene which is more carcinogenic locally than N-acetoxy-AAF, suggest that reaction of N-aryl-N-acetylnitronium ions with adenine in nucleic acids may be more closely related to the carcinogenicitiy of the parent compounds than is reaction with guanine.[19]

The observation that enzymatic synthesis of N-acetoxy-AAF has been demonstrated only in systems containing N-hydroxy-AAF, H_2O_2, and peroxidases led to testing of specific peroxidase inhibitors on the formation of arylamine-nucleic acid conjugates.[20] Conjugate formation was found to be insensitive to cyanide and

sulfide, demonstrating that the peroxidase-H_2O_2-induced formation of N-acetoxy-AAF, as reflected by the combination of N-hydroxy-AAF with nucleic acids, does not result from a peroxidase activity as measured by conventional assay techniques. Activation of N-hydroxy-AAF may be the result of an enzymatic activity distinct from previously recognized functions of the peroxidase molecule. Formation of reversible complexes of peroxidase with hydroxamic acids has been reported.[21] If analogous complexes of peroxidase-N-hydroxy-AAF, and of peroxidase-cyanide-N-hydroxy-AAF are formed, both of these complexes may be capable of catalyzing the activation of this hydroxamic acid.[20]

Conflicting results have been reported concerning the effect of N-hydroxy-AAF on nucleic acid synthesis in rat liver. Since the discrepancies reported may have been due to unavoidable variables present in vivo, an attempt to resolve the question was made by conducting the studies on slices from normal and regenerating rat liver.[22] In this system, N-hydroxy-AAF was equally inhibitory to RNA synthesis in both normal and regenerating liver. Inhibition of DNA synthesis in regenerating liver was found to parallel that of RNA synthesis. DNA synthesis in normal liver was not significantly inhibited at any concentration of N-hydroxy-AAF studied. The differential effect of the carcinogen on DNA synthesis would be explainable if the DNA synthesis in normal liver was occurring in cells other than hepatocytes and if these cells lacked the sulfotransferase necessary for activation of N-hydroxy-AAF to AAF-N-sulfate. There is some evidence to support this explanation.[22]

The recognition that animal cells possess mechanisms similar to those found in bacteria, by which they can repair certain types of physical and chemical damage to their DNA has intensified interest in the role of DNA repair in carcinogenesis. The finding[23] that patients with the hereditary disease xeroderma pigmentosum (XP) are deficient in repairing UV-induced damage to their DNA has suggested that this deficiency may account for their extraordinary susceptibility to skin cancer on sun-exposed areas of the body. The defect in typical XP cells is apparently in an endonuclease activity.[23] More recently, another human disease has been found to be associated with a different DNA repair deficiency. Cells from a patient with Hutchinson-Gilford progeria failed to show evidence of normal DNA strand rejoining in vitro after exposure to ^{60}Co gamma radiation.[24] Unirradiated progeric cells appeared to synthesize DNA normally, indicating that either: (1) the ligase involved in normal DNA synthesis and the ligase required for rejoining radiation-induced strand breaks must differ; or (2) other enzymatic steps necessary to produce the correct ligase substrate or to allow ligase-substrate contact must be deficient or greatly reduced in these cells.

Somatic mutation has often been suggested as a possible cause of both cancer and the changes which accompany aging. The fact that XP and progeria, one associated with almost 100 percent incidence of cancer and the other characterized by premature aging, have demonstrated defects in DNA repair supports this suggestion. Moreover, many, if not all of the known activated forms of chemical carcinogens are also mutagens.[25] N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) has been shown to mutate selectively the replicating fork of bacteria, thus giving rise to higher mutation yields in dividing bacteria than in those in which DNA synthesis has been completed.[26] To determine

whether malignant transformation by MNNG also is influenced by the stage of the cell cycle, transformation was studied in synchronized cultures of mouse fibroblasts.[27] MNNG-transformation showed marked cell cycle dependency, with the most sensitive phase being between 4 hours prior to S and the G_1-S boundary. One possible interpretation of this result is that the cells treated in late G_1 or early S phase may be unable to repair MNNG-induced damage and are transformed, while cells treated later in the cycle are able to repair the damage during the succeeding G_1 phase.

To date few studies have been reported on any aspect of DNA repair in the intact organism and in none of these studies was actual repair of DNA demonstrated by re-formation of broken strands. A method for measuring damage and repair of liver DNA in vivo has been developed and utilized to study repair of methyl methanesulfonate damage to rat liver DNA.[28] Results of this study showed that the time scale for induction of both DNA damage and DNA repair in liver is quite different from that in various cells in culture: damage and recovery in culture occur in minutes and hours rather than in hours and days as was found in liver. An accompanying report[29] presented the results of a comparison of DNA damage and repair in rat liver after administration of various methylating agents. N-methyl-N-nitrosourea (MNU), although noncarcinogenic for liver, caused single-strand breaks in liver DNA. Repair of damage to DNA by MNU was complete in the first week while that induced by the hepatocarcinogens dimethylnitrosamine (DMN) and methylazoxymethanol acetate (MAM) was still not complete within 14 days after administration of the compound.

The correlation between carcinogenicity and prolongation of DNA repair did not hold true for three cyclic nitrosamines examined.[30] 4-N-nitrosomorpholine (NM), a potent liver carcinogen, resembles DMN and MAM in that repair of the single-strand DNA damage requires at least 14 days. Single-strand lesions induced by the hepatocarcinogens N-nitrosopiperidine (NPD) and N,N-dinitrosopiperazine (DPZ), however, were repaired within 6 days. The basis for the differences in duration of repair are unknown. Among the three carcinogens tested in this study, only NM induced double-strand as well as single-strand DNA breaks. The double-strand breaks were rapidly repaired. The potent hepatocarcinogen 1-nitroso-5,6-dihydrouracil (NO-DHU) also induced both single- and double-strand breaks in rat liver DNA both of which are apparently repaired within 4 hours.[31] It is difficult to envisage a repair mechanism for double-strand DNA breaks in non-proliferating cells that does not introduce a high probability of error. Thus, the rapid repair of NO-DHU-induced lesions is consistent with the hypothesis[32] that tumor initiation may involve induction of error-prone breaks.

DNA repair synthesis has been measured in the livers of rats fed the hepatic carcinogen 3'-methyl-4-dimethylaminoazobenzene.[33] The maximum DNA repair synthesis detected occurred 3 hours after ingestion of the carcinogen; low levels of repair synthesis were still detectable at 16 hours after feeding. The relatively long delay in repair of single-strand damage observed with some carcinogens may then serve only to enhance the probability of occurrence of double strand breaks by increasing the opportunity for cell proliferation.[31]

Most known DNA repair mechanisms require synthesis of new DNA to replace the damaged segment. Photoreactivation is the specific repair of ultraviolet-induced cyclobutyl pyrimidine dimers in DNA by returning the pyrimidine moieties to their original configuration rather than excising and replacing them. A photoreactivating enzyme has been demonstrated in bacteria and certain vertebrates including primitive mammals. Such an enzyme has been thought to be absent in cells from placental mammals. A photoreactivating enzyme has now been demonstrated in human leukocytes and has been purified to apparent homogeneity.[34] The specificity of the enzyme for cyclobutyl pyrimidine dimers may allow direct evaluation of the role of these dimers in ultraviolet-induced human tumors.

VIRAL CARCINOGENESIS

During the past several years there has been an increasing number of reports concerning the apparent viral origin of several types of human cancer. RNA-containing particles associated with an RNA-dependent DNA polymerase have been detected in human tumors, the animal homologs of which are known to be induced by RNA viruses. Similar particles have recently been found in 30 of 49 human brain tumors examined.[35] No such particles were observed in nontumorous human brain tissue. Spontaneously occurring brain tumors in nonhuman species are reportedly rare[36,37] and no suitable animal model or corresponding animal virus is known.

In two sets of identical twins examined, the leukemic member of each set had particle-related sequences in the DNA of his leukocytes that could not be detected in the leukocytes of the healthy identical sibling.[38] This finding implies that the additional leukemia-specific information must have been inserted subsequent to fertilization, and argues against any etiologic concept that invokes vertical transmission of this information. A similar conclusion can be reached concerning the tumor-specific information in Burkitt's lymphoma and Hodgkin's disease cells. Unique sequences found in the DNA of Burkitt's and Hodgkin s tissues were not found in normal lymphoid cells.[39] The sequences in the two tumor types were related to each other but not to the DNA of Epstein-Barr virus which is associated with Burkitt's lymphoma.

A virus serologically indistinguishable from the Epstein-Barr (EB) herpesvirus is believed to be the causative agent of infectious mononucleosis (IM). To investigate further the relationship between viruses from the two diseases, DNA from EB virus isolated from Burkitt's lymphoma cells was hybridized in vitro with herpesvirus DNA from IM patients.[40] The thermal stability of the homologous and heterologous reassociated DNA molecules could not be differentiated, indicating at least 97 percent matching of base pairs between the DNAs obtained from the two sources and limiting the extent to which differences in viral genome could be responsible for the two disease states.

Association of a presumptive tumor virus with additional, non-neoplastic diseases is not limited to EB virus. A virus believed to be a variant of

SV40 has been isolated from frozen brain tissue of two patients with progressive multifocal leukoencephalopathy.[41] Nine of eleven electrophoretic fragments of the viral DNA co-migrated with DNA of a laboratory strain of SV40;[42] the two regions which differed are the same as those in which variation is seen with stable SV40 plaque morphology variants. The RNA virus believed responsible for feline lymphosarcoma also shows a striking association with feline infectious peritonitis and certain nonresponsive anemias.[43] Experimentally, the virus has produced thymic atrophy in kittens, which is accompanied by a fatal, ill-defined condition clinically similar to the naturally occurring "fading kitten syndrome".[44] The reason for the differing pathological effects of the feline virus (if, indeed, all of these conditions are due to the same virus) is not known. In the case of the EB virus, however, there is evidence that Burkitt's lymphoma may result only when EB infection is accompanied by co-infection with a specific RNA virus. The particles with morphological and biochemical similarities to RNA tumor viruses which have been found in Burkitt's tumors[45,46] have not been found in cells from IM patients.[46] To explore the possibility of an interaction between these viruses and the EB virus, experiments were performed[47] with an animal model, Marek's disease, a malignant lymphoproliferative disease associated with a herpesvirus. Controlled contact studies demonstrated that tumorigenesis in a line of isolator-derived, barrier-sustained, specific pathogen-free chickens requires exposure to both the Marek's disease herpesvirus and an avian leukosis RNA virus, RAV-2. Conventionally raised chickens showed a significant enhancement in tumor occurrence after infection with both viruses as compared with birds similarly infected with either virus alone.

The genomes of both DNA and RNA tumor viruses are apparently integrated into the host cell DNA, either directly in the case of DNA viruses or in the form of a DNA provirus in the case of RNA tumor viruses. Analysis of the relationship between this integrated viral genetic information and the development of malignancy is complicated by the finding that tumor virus-specific DNA can be found inserted in the DNA of some nonmalignant cells. In a recent study, virus-related DNA sequences were determined before and after transformation of marmoset fibroblasts by RNA tumor viruses.[48] Cells transformed by non-indigenous tumor viruses (Rous sarcoma virus and feline sarcoma virus) acquired viral DNA sequences not present in normal cells. The new subsequences were always hybridizable with the transforming virus. In contrast, the total DNA product of the indigenous simian RNA tumor virus, SSV-1, annealed with the DNA of normal marmoset fibroblasts as well as with SSV-1-transformed cells. Only limited homology (5 percent) was observed between the DNA of uninfected marmoset cells and the DNA products of RSV and FeSV.

DNA sequences complementary to the RNA of avian myeloblastosis virus (AMV) and other avian leukosis viruses are present in all apparently normal chicken cells examined thus far. Experimental infection of chicken cells with AMV revealed, however, that the resulting transformed cells contained twice the level of AMV-specific DNA sequences observed in normal chick embryo cells.[49] Further studies revealed that the transformed cells contained new AMV-specific DNA sequences not present before infection. There are two classes of virus-specific DNA sequences, distinguished by the rapidity of their

hybridization with viral RNA. Both classes were increased in transformed chicken cells. Rat cells transformed by avian sarcoma virus B-77 acquired only the slowly reacting sequences; normal rat cells did not contain DNA that hybridized with AMV RNA. It is therefore possible that the slow-reacting DNA sequence may contain the information necessary for oncogenesis.

Genetic recombination between endogenous and exogenous avian RNA tumor viruses has been demonstrated.[50] Recombination was obtained between the genomes of chick helper factor (chf) and nondefective strains of Rous sarcoma virus (RSV), including temperature-sensitive strains. Recombinants of chf with the defective Bryan strain RSV could not be isolated. A stable defective mutant similar to the Bryan strain has recently been isolated from nondefective Schmidt-Ruppin RSV and available evidence indicates that a deletion mutation is involved.[51] The demonstration of recombination between nondefective strains of avian tumor viruses raises the question of whether RSV can acquire other species of host messenger RNA of the appropriate size.[50] Acquisition of host nucleic acid sequences has been observed with DNA tumor viruses. Serial passage of SV40 in monkey kidney cells, for example, resulted in accumulation of closed-circular SV40 DNA molecules containing covalently linked sequences homologous to host cell DNA.[52,53] The phenomenon of host substitution in the viral genome may be a general feature of DNA tumor viruses since plaque-purified isolates of polyoma virus also generate substituted progeny after serial undiluted passage in primary mouse kidney cells (unpublished observations cited in reference [52]). Vaccinia virus degrades HeLa cell DNA early during productive infection but does not appear to incorporate the host DNA or to re-utilize its components.[54]

The relationship of viral DNA synthesis to host cell DNA synthesis and the origin of the enzymes involved in these processes are topics of vital importance to an understanding of viral carcinogenesis. There are three separate avian systems with RNA-dependent DNA polymerases: virions of avian leukosis-sarcoma viruses, virions of reticuloendotheliosis viruses, and a fraction from normal chicken cells.[55] The serological relationships among these polymerases and between these and two DNA polymerases from chick embryos have been investigated.[55] The two virus-associated enzymes are not serologically related to each other or to either of the embryonic polymerases or to the endogenous chicken reverse transcriptase. The endogenous enzyme was at least partially neutralized by antibody against the small DNA polymerase isolated from chick embryos. These studies utilized unpurified polymerases and it is possible that purification will reveal relationships which are obscured at present. The soluble DNA polymerase of murine RNA tumor viruses appears to differ from that of avian tumor viruses in its lack of detectable RNase H activity and in its relatively low template activity for viral RNA and natural (salmon) DNA.[56]

The small DNA tumor viruses such as polyoma and SV40 are believed to utilize cellular enzymes for replication of their genomes.[57] A lysate from polyoma-infected mouse cells has been used to investigate polyoma viral DNA synthesis.[57] Synthesis was semiconservative and proceeded, at least in part, by a discontinuous mechanism, raising the possibility that more than one

DNA polymerase may be involved in chain growth. Covalent association of RNA with the small, discontinuously growing DNA fragments has been demonstrated.[58,59] Polyoma viral DNA synthesis is apparently initiated by a short sequence of ribonucleotides on the 5' end.[58] Studies of SV40 DNA replication also indicate the involvement of two different DNA polymerases.[60] SV40 DNA apparently replicates by a discontinuous mechanism similar to that observed for polyoma virus.

Analysis of cell transformation by mutants of polyoma virus has shown that several aspects of transformation are under the control of viral genes. The recent isolation of a temperature-sensitive mutation affecting the transforming ability of SV40 has made it possible to extend these findings to SV40 transformation.[61] Infection of rat cells with ts-640, a temperature-sensitive SV40 mutant, resulted in transformation at 33°C but not at 40°. The cells transformed at 33° did not revert to normal when shifted to 40°, indicating that the viral gene function under study may be required for the initiation but not the maintenance of transformation. In productive infection of monkey kidney cells, ts-640 was defective in its ability to synthesize infectious viral DNA and infectious viral particles at the restrictive temperature. The affected gene function is required for normal replication of viral DNA, suggesting that a common step may be present in the process of viral DNA replication in productive infection and that of cell transformation in nonproductive infection. Morphological transformation of rat kidney cells by a strain of murine sarcoma virus of the MSV-Moloney leukemia virus complex was found to be cold-sensitive.[62] Cells transformed by this virus expressed their transformed phenotype at 39°C but not at 33°. This is believed to be the first report of a cold-sensitive tumor virus mutant. Maintenance, as well as initiation, of the transformed state appeared to be dependent on the cold-sensitive viral function.

Progressive inactivation of SV40 by ultraviolet light has shown that transformation ability is apparently the most resistant viral function.[63] T-antigen induction paralleled transforming ability. After 5,000 to 10,000 ergs/mm^2 UV doses, transforming capacity in Syrian hamster kidney cells was enhanced 180 percent for "complete" SV40 and 270 percent for "defective" SV40. A simultaneous increase in T-antigen induction was observed.

In the same cell system a host cell repair mechanism was demonstrated which is to a large extent responsible for the high resistance to UV inactivation of the transforming capacity of SV40.[64] The differences between the rates of inactivation of the capacity for infectivity and transformation of UV-irradiated SV40 which persist in the presence of caffeine may reflect differences in the size of the viral genome utilized for replication and transformation; alternatively, they could reflect different degrees of cell repair of UV damage in the two cell systems.

It is not known whether the SV40 T-antigen is virus-coded or coded by the cell genome as a result of virus integration into the cell DNA. Although this question remains unanswered, it appears that expression of the antigen in human-mouse hybrid cells is associated with retention of the human C-7 chromosome.[65] Comparative determination of the number of SV40 genome

equivalents in the hybrid cells and in SV40-transformed human cells may resolve the question of whether the viral genome is integrated specifically in the C-7 chromosome or whether it is integrated in several chromosomes with expression of T-antigen associated only with integration in C-7.

Studies with Rous sarcoma virus (Bryan strain (RAV-1)) in chick embryo fibroblasts indicate that the role of the proviral DNA extends beyond induction of morphological conversion and that an intact provirus is required for maintenance of the transformed state.[66] Light-inactivation of bromodeoxyuridine (BUdR) sensitized proviral DNA indicates that no irreversible process leading to morphological conversion is initiated by a non-integrated or by an integrated provirus. Three days after infection, when proviral DNA is integrated in 100 percent of the cells, exposure to visible light of cells infected in the presence of BUdR results in the suppression of about 50 percent of the foci, indicating that the provirus can be inactivated after integration occurs.

The template properties of chromatin and DNA isolated from SV40-transformed mouse cells were compared.[67] The SV40 sequences in the RNA transcribed from chromatin in vitro and in RNA isolated from the transformed cells were analyzed by hybridization to radioactive RNA transcribed from SV40 DNA. The results indicate that the same sequences of the SV40 "minus" strand are transcribed in vitro from chromatin as are found in transformed cells. When DNA isolated from the chromatin is used as a template the entire "minus" strand is transcribed. This finding indicates that chromatin as a template for E. coli RNA polymerase allows transcription of only those sequences transcribed in vivo and is thus appropriate for study of gene regulation in cell-free systems.

CANCER BIOLOGY AND BIOCHEMISTRY

The abnormal growth characteristics exhibited by cancer cells are not necessarily due to genomic gain or loss although these mechanisms may be operative in specific cases. Differential gene transcription can account for both gain and loss of heritable properties in malignancy as in normal differentiation. Several recent studies have compared the extent and specificity of transcription in normal and malignant cells and others have investigated the factors which regulate transcription in both types of cell.

The genomes of eukaryotic cells contain repetitive and non-repetitive nucleotide sequences. Nucleic acid hybridization was used to determine the extent to which non-repetitive DNA is transcribed in normal and virus-transformed mouse cells.[68] The results showed that the extent of transcription of the unique DNA sequences, although greater in transformed than in normal cells, does not vary in either case between subconfluent and confluent cultures. Normal cells, however, show some variation in the sequences expressed between the two growth states whereas transformed cells do not.

Regulation of gene transcription occurs at various levels, the most direct of which is mediated by either permanent or periodic association of certain

DNA segments with chromatin proteins. The synthesis of non-histone chromosomal proteins has been studied as a function of cell-cycle position in synchronized cultures of Chinese hamster ovary cells.[69] Although non-histones were synthesized in all phases of the cell cycle examined, non-histone synthesis was three- to five-fold higher in late G_1 compared with the other phases. The increased synthesis was characterized by production of polypeptides larger than 45,000 daltons. Cells blocked in late G_1 by hydroxyurea continued to synthesize non-histones at high rates. Removal of hydroxyurea was followed by a dramatic decrease in synthetic rate, suggesting that synthesis of high molecular weight non-histone chromosomal proteins is coupled to DNA synthesis.

The functional presence of gene regulator proteins obviously depends on their catabolism as well as on their synthesis. The catabolism of nuclear proteins was therefore compared in control and phytohemagglutinin (PHA)-stimulated normal human lymphocytes and lymphocytes from patients with various types of leukemia or Burkitt's lymphoma.[70] Unstimulated normal and leukemic lymphocytes appeared to lack nonhistone protein electrophoretic bands corresponding to the higher molecular weight regions of the gels. Homologous fractions from PHA-stimulated normal lymphocytes and Burkitt's lymphoma cells contained the high molecular weight protein bands. Nonstimulated normal and leukemic lymphocytes were found to contain a nuclear protease capable of markedly degrading proteins in Burkitt's lymphoma cell nuclei. These enzymes may therefore have an important role in regulating the content and function of nuclear proteins.

The transcriptional capacity of chromatin from two transplantable rat hepatomas has been compared with that of normal liver.[71] Chromatin from the slowly growing, highly differentiated Morris hepatoma 9618A had the same in vitro template activity for RNA synthesis as did chromatin from normal rat liver and the electrophoretic pattern of the nonhistone proteins was qualitatively identical with the pattern obtained with liver nonhistone proteins. It did, however, have an altered content of nonhistone protein and RNA, an altered rate of amino acid incorporation into histone protein, and an altered turnover of the nonhistone proteins. Chromatin from the rapidly growing Morris hepatoma 5123C exhibited a marked increase in template activity, compared with that of normal liver and there were several alterations in the electrophoretic pattern of the nonhistone proteins. The data suggest that some of the differences between chromatin of rapidly growing hepatomas and normal liver are a feature of malignant progression rather than of neoplastic transformation.

Several macromolecules in addition to nuclear proteins are known to affect gene transcription. Several hormones have been shown to exert their effects on target tissues by affecting transcription, often through the mediation of cyclic nucleotides. Ovarian hormonal regulation of rapidly labeled RNA synthesis has been investigated in normal, preneoplastic, and neoplastic tissues of BALB/c mouse mammary glands.[72] Administration of the hormones to intact or ovariectomized or adrenalectomized virgin mice induced a sharp rise in rapidly labeled RNA synthesis with patterns like those observed during pregnancy. Preneoplastic nodules in virgin mice actively synthesized rapidly

labeled RNA regardless of their ultimate tumorigenicity; ovariectomy, adrenalectomy, and hormone administration did not affect RNA synthesis in the nodules. Results with nodule-derived tumors were similar. The results suggest that sustained lobulo-alveolar structures and the hyperactive growth patterns of the preneoplastic nodules may be related to altered hormonal control of the transcriptional activity of the nodule cell genome but that this metabolic abnormality is not the ultimate carcinogenic event in the mammary cells.[72]

The lack of hormonal responsiveness observed in many types of tumors is apparently due in certain cases to alterations in the cell surface which affect hormone receptor sites. The sialyl transferase of disialoganglioside formation is depressed in mammary tumors induced in the rat by 7,12-dimethylbenz[a]anthracene.[73] This aberration is potentially important in view of the known role of carbohydrate residues in imparting specificity to surface membranes. Two rat mammary carcinomas and one from the mouse have recently been tested for prolactin binding capacity.[74] The 7,12-dimethylbenz[a]-anthracene-induced rat tumor, which is dependent on prolactin for growth, exhibited a range of binding activity which varied between 30 and 80 percent of the lactating mammary gland. The R3230AC rat tumor, which is prolactin-responsive for milk protein synthesis but not for growth, had approximately 15 percent of the prolactin receptor activity of lactating mammary gland. The C3HBA mouse tumor, a relatively autonomous tumor, had no detectable prolactin receptor activity.

Most studies of hormonal regulation of proliferation and function in epithelial cells have been performed in cells from nonhuman species. A recent study[75] has shown that in contrast to results obtained with mammary cells from several other species, DNA synthesis in human mammary gland cells in vitro is, to a considerable extent, not dependent on hormonal or serum factors. The cells used in these studies were apparently normal although they were obtained from tissue removed because of the presence of an associated carcinoma.

Externally induced changes in the cell surface are known to play an important role in regulating cell proliferation, differentiation, and behavior. Contact inhibition of growth is the property in vitro whose loss is most closely correlated with tumorigenicity in vivo. A stable, contact-inhibited cell line has been derived from a highly malignant hamster melanoma cell line. The contact-inhibited line produces a diffusible factor which restores contact inhibition of growth to the malignant cells.[76] Another diffusible substance, from embryonic mouse mammary mesenchyme, can induce differentiation in a mouse mammary tumor in vitro.[77] The tumor, adenocarcinoma BW 10232, was cultured with the embryonic tissue but separated from it by a Millipore filter. Tubules developed in the tumor; DNA synthesis declined; and a presumptive acid mucopolysaccharide matrix, not evident in the controls, appeared.

Cyclic nucleotides are currently among the most intensively studied regulators of cell behavior. Rat hepatic adenyl cyclase stimulation by glucagon and epinephrine shows an abrupt change in the energy of activation at 32°C.[78] The energy of activation for the cyclase reaction above 32° is about 1.7 times that found below this temperature. Cyclase activity stimulated by fluoride,

prostaglandin E_1, or 1-propanol, or activity in the absence of added stimulators did not show this change. In view of the relatively large mole fraction of cholesterol present in rat liver plasma membrane (which appears to inhibit phase transitions in bulk membrane lipids), it is suggested[78] that the thermal sensitivity resides in protein rather than lipid components or that the cyclase is restricted to cholesterol-poor membrane regions.

The growth rate of a mouse plasma cell tumor (MPC-11) in suspension culture is inhibited by cyclic AMP. The effect is concentration-dependent and is readily reversible by addition of insulin.[79] The inhibitory effect of cyclic AMP on cell growth has been observed in a wide variety of cell types. The action of cyclic GMP appears antagonistic to that of cyclic AMP and the inhibitory effects of the latter on mitosis in cultured mouse cells can be counteracted by cyclic GMP.[80] Nontransformed mouse fibroblasts can also be stimulated by cyclic GMP to undergo transition from a resting to a growing state.[81] Thus, cyclic GMP may act as a positive signal, and cyclic AMP as a negative signal, for controlling cell growth in vitro.

Cyclic AMP incorporated in the growth medium of XC rat sarcoma cells blocks their ability to form syncytia, decreases their agglutinability with concanavalin A, and alters their plasma membrane structure.[82] Studies[83] of these cyclic AMP-induced phenomena strongly indicate that the cell surface modifications require de novo messenger RNA synthesis which is associated with an increase in DNA-directed RNA polymerase II activity. The facts that cell surface modifications by cyclic AMP depend on mRNA transcriptase are reversible and short lived,[82] and are closely related to the cell cycle of the normal cell[84], suggest that cell division may be regulated by the relative transcriptional activity of one or more genes continuously producing mRNA, which govern properties of the cell surface.

Abnormalities in the structure and composition of cancer cell surfaces are well documented and have, in many cases, been shown to correlate well with malignancy. Cells from a cancerous epithelial cell strain derived from a rat liver tumor have been shown to make defective intercellular junctions so that there is no communication between cell interiors.[85] Fusion of these cells with either normal liver cells or normally growing fibroblasts derived from the tumor resulted in simultaneous correction of the junctional defect and appearance of normal growth characteristics in the heterokaryons and in their hybrid (mononuclear) descendants.[86]

Many transformed cells and protease-treated normal cells agglutinate at much lower concentrations of lectins than do homologous normal cells. One of the earliest models suggested to explain this phenomenon was the "cryptic site hypothesis,"[87] which suggested that the agglutinin receptor sites are exposed on the transformed cell surface but are buried in the normal cell surface (where they can be exposed by proteases). Results of subsequent work, including studies of the binding of ^{3}H-concanavalin A (conA) to normal and transformed cells[88], indicated that this model required substantial revision. The finding that ferritin-labeled conA is clustered on the surface of transformed cells, but is distributed randomly on untreated normal cells led to the proposal[89] that it was the distribution, rather than the number,

of available binding sites which characterized transformed cells. More recently, other investigators have proposed[90] on the basis of additional findings, that the conA molecule is itself responsible for the observed clustering of the label as opposed to the above model in which the binding sites are viewed as clustered before the addition of conA. According to the later model the following steps occur: (a) One site of a multivalent conA molecule binds to one of the randomly distributed receptor sites on the transformed cell surface, (b) A second binding site of the same conA molecule traps a second conA receptor which is moving around within the same cell surface, thereby initiating the formation of a cluster of the conA receptor sites. Factors which limit the fluidity of the plasma membrane might prevent the movement of conA receptor sites within the plane of the surface and thereby inhibit the agglutination process. Such a step could explain the temperature sensitivity[90] of the agglutination reaction, the 15°C transition temperature[90] for agglutination and the inhibitory effect of glutaraldehyde[90] on agglutination. (c) Once the clustering of receptor sites has begun, it continues because of the interaction of the multivalent conA molecules with the multivalent receptor sites until all available sites are stably cross-linked by agglutinin molecules.

A cell membrane component which has received increasing study in the past several years is cholesterol. A carcinogenic derivative of cholesterol, cholesterol α-oxide, has been demonstrated in UV-irradiated skin of humans[91] and hairless mice.[92] The time course of cholesterol α-oxide formation in the skin of hairless mice receiving chronic suberythemic levels of UV radiation suggests that it may be involved in UV-induced carcinogenesis.[93] Formation of cholesterol crystals can occur in response to virus infection[94] although thus far this observation does not appear related to viral carcinogenesis.

Most studies of cholesterol in cancer cells are concerned with regulation of cholesterol synthesis rather than with its role as a possible carcinogen precursor or its function in cell membrane. In normal tissues, active tissue growth appears to be characterized by heightened cholesterogenesis (reviewed in reference [95]). An apparent lack of responsiveness to feedback inhibition of cholesterol biosynthesis has been observed in hepatomas of several species and in other tumor types as well; some tumor cells have also been found to exhibit an apparent surface defect which impairs their uptake of cholesterol, thus preventing interaction of cholesterol with intracellular regulatory sites (for details and references see NCI Extramural Activities Annual Report Appendices for fiscal years 1971, 1972 and 1973). Since normal lymphatic cells possess a low sterol synthetic rate, inhibition of sterol synthesis is difficult to demonstrate in these cells. However, the cholesterol feedback mechanism did appear to be present in spleen cells from mice with spontaneous leukemia although the inhibited values from leukemic cells were still five to six times higher than those of normal controls.[96] Consistent with its function as the regulatory enzyme for de novo sterol synthesis, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity was elevated in the leukemic spleens. Cells from patients with familial hypercholesterolemia have been found[97] to have a defect in the regulation of HMG-Co A reductase which was later shown[98] to result from a decreased capacity to bind the low density lipoproteins which normally suppress the synthesis of this enzyme. The defect is believed to reside in the cell membrane.[97]

The effects of dietary cholesterol on bile acid synthesis have been compared in normal liver from tumor-free rats and in the Morris rat hepatoma 3924A.[99] Several molecular species of bile acids were synthesized by the tumor, constituting what is believed to be the first example of bile acid synthesis found in a malignant tumor. Bile acid production from acetate is suppressible by dietary cholesterol in normal liver but in the tumor neither this pathway nor that involving mevalonate was affected by dietary cholesterol.

These results are consistent with the reported deletion of the dietary cholesterol feedback system in hepatomas. Cholesterol metabolism in the livers of rats treated with the carcinogen 2-acetylaminofluorene (AAF) appears to be similar in several respects to that in transplantable hepatomas. Cholesterol uptake, storage, and synthesis have been investigated in the livers of AAF-treated rats.[100] Analysis of the results of this study suggested that in the early precancerous liver there may actually be no defect in the entry of cholesterol into the cell, but rather a defect in an intracellular cholesterol binding or storage mechanism. Such a defect could lead indirectly to an apparent decrease in the entry of ^{3}H-cholesterol into the cell. Similar studies of hyperplastic liver nodules and primary hepatomas induced in rats by AAF have shown[101] that these systems take up dietary cholesterol at a rate only slightly less than that in normal liver, i.e. 10- to 30-fold greater than that reported for transplantable hepatomas. This finding emphasizes the danger of relying only on results obtained from transplantable tumors, which may be unrelated to the situation in primary tumors simply because of such factors as alterations in the blood supply. Transplantable hepatomas have an arterial blood supply while normal liver and probably primary hepatomas[102] derive up to 75 percent of their blood from the portal vein.

Studies of cholesterol regulation in human cells should be considerably facilitated by the recent finding obtained from hybridization of human cells with mouse L cells. The latter synthesize desmosterol as their principal sterol, presumably because of a defective or absent sterol Δ^{24}-reductase.[103] Analysis of specific chromosome retention by the hybrids showed that retention of human chromosome F-20 was associated with the ability to convert desmosterol to cholesterol.[103]

HOST-TUMOR INTERACTIONS

Immunological factors play an important role in resistance to cancer. Tumor-specific or tumor-associated antigens have been found on tumor cells, and cell-mediated and humoral immune responses to these antigens have been demonstrated. The various cell populations involved in immune processes are being characterized and information is rapidly accumulating concerning the role of each of these cell types in the immune response.

Lymphocytes can be classified as thymus-derived (T cells, involved in cellular immunity) and bone marrow-derived (B cells, involved in humoral immunity) according to origin, surface characteristics, and function. The scanning electron microscope (SEM) is being used to identify T and B cells and to compare their surface architecture. The distinction between these lymphocytes on the basis of their surface structure can be made by SEM with ease

in most but not all instances. About 80 percent of peripheral blood lymphocytes in a recent study[104] were T cells which appear to be smaller, relatively smooth cells while about 20 percent of the cells were B type which appeared to have a complex villous surface pattern. A small population, about 1-2 percent, possessed both T and B markers. Five of six cases of chronic lymphocytic leukemia (CLL) were of B cell origin while one case had B and T cells in equal numbers. In both the T and B rosetting systems, the points of attachment to red blood cells are the microvilli.[105]

In another study,[106] morphologic aberrations of protein-synthesizing organelles within B lymphocytes of two patients with CLL correlated with functional immunologic deficits manifested by failure to differentiate to mature secretory cells following mitogenic stimulation. T cell surface markers have been found[107] on lymphoblasts in the bone marrow from one of four children with untreated acute lymphocytic leukemia (ALL). Initial clinical characteristics of this patient suggested a more aggressive disease. B and T lymphocytes were found in the blood and bone marrow of 24 children with ALL in remission. Another study showed no quantitative changes of T-cell population in children with acute lymphoblastic leukemia regardless of activity of disease and chemotherapy. However, it was shown that absolute lymphocyte count decreased, suggesting that the T-cell is resistant to chemotherapy.[108]

The leukemic cells of a patient with ALL contained a DNA polymerase which has the properties of a terminal deoxynucleotidyl transferase, an enzyme found only in thymocytes.[109] These cells also contain the three major DNA polymerases found in growing cells. The ALL cells appear to be thymic cells blocked very early in their differentiation. A hypothesis[110] has been proposed that the molecules which confer specificity to T cells may be similar to, if not identical with, the immunoglobulins produced by B cells. The hypothesis further postulates that the terminal transferase acts as a somatic mutagen to diversify the amino acid sequence in the variable region of T cell surface immunoglobulin molecules and thus produces T cell somatic diversification.

Kinetic studies of lymphocyte transformation have led to the hypothesis that in Hodgkin's disease circulating T cells are stimulated by the presence of active disease.[111] This stimulation of T cells leads to circulating T cell depletion and to an increase in the number of cells circulating that are in active DNA synthesis. The degree of impairment of cell-mediated immune reactions would then depend upon the degree of T-cell depletion. Serum antibody responses usually appear normal in these patients. B cell activity has been assessed[112] in a group of patients with Hodgkin's disease by measuring in vitro splenic IgG synthesis. Augmented splenic IgG production occurred in 20 of 22 patients. Supernatant IgG appeared to interact specifically with homologous lymphocytes, suggesting that the spleen in Hodgkin's disease may respond with a humoral antibody to some antigen associated with lymphocytes.

Transplantation into hamsters of lymphoid cells from humans with lymphoid cancer or infectious mononucleosis has resulted in growth of malignant

tumors of two types, those derived solely from subjects with ALL secondary to lymphosarcoma which progressed to leukemia and did not secrete immunoglobulin and those that secrete immunoglobulin and do not progress to acute leukemia. The two tumor types may be derived from T and B lymphocytes, respectively. Thus, heterotransplantation studies may be useful in relating diseases to disturbances of the B and T cell populations.[113] T cells appear to participate in lymphocyte trapping,[114] the process of lymphoid cell flow and accumulation during an immune response. In an experimental mouse tumor system, the kinetics of lymphocyte trapping correlated with the growth of the tumor and maximal retention of lymphocytes corresponded to the time of tumor regression.[115]

Two in vitro assay systems have been used[116] to separate and characterize the cells involved in cell-mediated homograft rejection. The mixed leukocyte culture (MLC) technique tests lymphocytes' ability to recognize foreign antigens and the cell-mediated lymphocytolysis (CML) assay measures cells involved in the killing phase of homograft reaction. Studies have suggested that two separate lymphocyte populations appear to be involved in these recognitive and destructive phases of cell-mediated immunity. Antiserum to β_2-microglobulin (β_2m) a low-molecular-weight protein constituent of lymphocyte membranes, blocks MLC reactivity as well as reactivity of lymphocytes to phytohemagglutinin but does not interfere to the same extent with T cell rosette formation with sheep red blood cells. These findings suggest a possible relation between β_2m and recognition units on the T cell surface.[117]

Macrophage surveillance requires the presence of opsonins or recognition factors (RF) present in the α-2-globulin fraction of plasma. The role of RF in neoplasia is being evaluated,[118] since loss of RF could contribute to a loss in immunologic tumor rejection mechanisms. Human RF administered to rats at the site of injection of Shay chloroleukemic cells plus glucan, an agent which activates reticuloendothelial cells, produced 86 percent suppression of tumor growth. These data suggest that macrophages and RF are important in tumor cell detection and destruction mechanisms. Lysozyme (LZM) appears to be released when macrophages react with target cells and recent data suggest that LZM may be a mediator of the anti-tumor functions of macrophages.[119] Homologous mammalian LZM had specific effects on the in vitro growth patterns and morphology of normal and transformed mammalian cells apparently because of effects on constituent membranes. It is postulated that the cellular effects of LZM are due to a combination of its cationic properties, facilitating interaction with the negatively charged cell surfaces and enzymatic effect on membrane polysaccharide(s). Thus LZM may be important in the regulation of certain membrane-dependent cell functions and may participate in the surveillance of membrane abnormalities.

Various assays of cellular immunity (lymphocyte transformation, macrophage migration inhibition, delayed hypersensitivity skin tests, MLC and CML) and serologic assays (primary and secondary antibody response to antigens, immunoglobulin levels, and serum factors responsible for blocking, potentiation and arming) have been developed to monitor the various parameters of immunologic reactivity of cancer patients and current investigations

are attempting to determine to what extent these tests correlate with one another and with the clinical course of the disease.[120-124] These measurements may be useful in assessing the disease state and in planning immunotherapy.

Recent advances in understanding the immunologic mechanism of tumor rejection and impairment of the host defense system have suggested that a depressed or absent immune response can be stimulated or restored. Non-specific stimulation of immune reactivity using Bacillus Calmette Guerin (BCG)[125] and Listeria monocytogenes[126] is being evaluated in various experimental animal models. Specific stimulation is being attempted by modifying tumor cell surface membranes to increase their immunogenicity. Firmly established transplantable C3H/HeJ mammary carcinomas could be inhibited by host challenge with Vibrio cholerae neuraminidase (VCN)-treated tumor cells.[127] The effect was totally immunospecific: VCN altered the immunogenicity of the unique tumor antigens, whereas VCN-treated tumors bearing shared mammary tumor virus antigen could not induce the regression. Repeated vaccination with modified tumor cells was successfully used to immunize mice to the P1789 lymphoma, a tumor-host system characterized by minimal natural immunologic responsiveness.[128] Several animal model systems are currently providing information on the mechanism of action of passive transfer of cellular immunity.[129,130] Specific antitumor antibodies conjugated to potent toxins such as diphtheria or ricin also offer a potential means of selective destruction of tumor cells.[131] In one study,[132] heterologous antitumor sera administered to mice with advanced ovarian cancer resulted in 30 percent long-term survival. It was suggested that the antibodies may have functioned as deblocking antibodies in this system.

Serum blocking factors removed from sera of rats tolerant to skin allografts appear to be comparable to those present in tumor-bearing animals and to have a similar mechanism of action. Either antigen alone or antigen and antibody complexes can block in vitro, and blocking can occur both at the effector cell and at the target cell level.[133] In another study IgA isolated from human sarcomas has been identified as a blocking immunoglobulin in an in vitro human sarcoma system.[134] Administration of combinations of methotrexate, 5-fluorouracil and Imuran, in the proper regimen, resulted in synergistic suppression of 19S antibody production in C3HeB/FeJ mice. Thus, drug combinations may prove useful in the control of serum blocking factors.[135]

The use of an in vitro assay which can differentiate between cytotoxic vs. blocking antibody has made it possible to predict enhancement of canine renal allografts.[136] The active immunoglobulin component responsible for the in vitro blocking was found in the IgG fraction. It is not clear whether blocking as well as cytotoxic activities reside in the same antibody molecule and it may be that the presence or absence of immunological enhancement depends on a balance among many factors. This method of detecting blocking antibodies may help to assess their role.

The athymic-nude mouse offers an animal model for studying the effects of the absence of thymus-dependent cell mediated immunity on tumor development. A spontaneous malignant lymphoma has been found for the first time in a

homozygous "nude" mouse surviving 156 days.[137] The latent period and incidence of local sarcomas or lung adenomas after perinatal exposure to methylcholanthrene was found to be similar in the immunologically normal nude heterozygotes and the immunologically deficient athymic-nude homozygotes.[138] These results do not support the theory that thymus-dependent immunity acts as a surveillance mechanism preventing tumor development.

Various tissue culture and animal models are being used to study the graft-versus-host (GVH) reaction. Morphologic and virologic analysis shows that replicating, infectious C-type virus particles are activated during the GVH and MLC reactions by a specific immunologic mechanism. It is not known whether these viruses cause the lymphomas that can develop during GVH reaction; bioassays to test for the oncogenicity of the viruses activated during MLC are in progress.[139] Many approaches are being tried to prevent, modify, or eliminate the GVH reaction. Studies in animal model systems show that Fab fragments of antilymphocyte globulin[140] and combination chemotherapy with procarbazine and cyclophosphamide[141] may offer useful treatment for GVH reactions. Antithymocyte serum given to dogs before histoincompatible marrow grafting and methotrexate after grafting prolonged survival but there was no effect on long-term survival.[142]

Experimental studies in dogs have been performed to answer questions regarding the mode of spread of Hodgkin's disease.[143] The direction of lymph flow in obstructed thoracic ducts was studied by following contrast material distribution by radiological and histological methods. Results indicate that flow in the intrathoracic portion of the obstructed duct is usually not reversed, suggesting that in Hodgkin's disease, retrograde flow via the thoracic duct is unlikely. However, retrograde lymph flow to paraaortic and presacral nodes from an infradiaphragmatic focus may be possible when thoracic duct flow is impaired.

The incidence of experimental metastasis is dependent on many factors including the tumor cell preparation and the quality of the tumor cell itself.[144] Experimental pulmonary metastases resulting from injection of B16 melanoma cells into mice were not determined by the number of viable tumor emboli but rather by the total number of and/or size of circulating emboli. Dead tumor cells injected with living tumor cells increased the incidence of metastasis. In addition, the incidence of metastasis could be significantly increased when low numbers of normal or sensitized lymphocytes were mixed with tumor cells while high numbers of immunized lymphocytes decrease the incidence of metastasis suggesting that weak immune stimulation was beneficial to cancer growth and dissemination.[145]

In a clinical study,[146] distant metastases were found in 96 (12 percent) of a total of 799 adult patients with epithelial tumors of head and neck. The lungs were the most frequent site followed by bone and liver but metastases to heart, spleen, and G. I. tract were frequently found. The largest number were found in patients with carcinoma of the nasopharynx and the smallest number in patients with carcinoma of the vocal cords. More than 50 percent of patients in whom the primary tumor was controlled developed metastases. Extensive primary tumors were the most likely to develop metastases. Two-year

survival was 25 percent, 5-year survival was 8 percent and almost 90 percent of the patients were dead within 2 years of the development of distant metastases.

Many studies are aimed at understanding the roles of hormones in the genesis of cancer and in the regulation of its growth. Several laboratories are engaged in studying the association between pituitary prolactin and mammary cancer. Daily treatment of mice with 2-bromo-α-ergocryptine (CB-154), an inhibitor of prolactin, markedly suppressed spontaneous mammary hyperplastic alveolar nodular development and virtually eliminated the appearance of mammary tumors.[147] Enhancement of DMBA-induced rat mammary tumor growth by high dietary fat may be mediated through alterations in circulating prolactin since CB-154 suppressed the formation of tumors.[148] In another study, estradiol-17β and to a lesser extent progesterone exerted a protective effect against DMBA and aflatoxin B_1-induced cytotoxicity in cultured liver cells. Other steroids were inactive.[149]

EPIDEMIOLOGY

The etiologic role of exogenous estrogens in malignant and benign breast disease is being actively investigated.[150] An epidemiologic study of benign breast disease in relation to oral contraceptive use was undertaken to determine whether it shares common characteristics with breast cancer. There were no differences in use of oral contraceptives or estrogen among patients with either cystic disease or fibroadenoma and controls. Fewer patients with cystic disease than controls had used oral contraceptives for more than one year. These data suggest that these steroid hormones have no role in the causation of benign neoplasms of the breast. In the cystic disease group, there was a deficit of postmenopausal women and women whose first pregnancy was under age 25. The late first pregnancy is a feature shared with breast cancer, but the absence of a difference in frequency of artificial menopause does not support an epidemiologic similarity between the two conditions.

Epidemiologic data have shown international differences in various etiologic factors in breast cancer. Histologic characteristics of tumors have now been compared[151] in breast cancer patients in Boston (high breast cancer rate) and in Tokyo (low breast cancer rate). Tumors of small-cell or invasive ductal histologic type and tumors with a high degree of fibrosis were more frequent in Boston than in Tokyo. There were more intraductal, medullary and colloid tumors in Tokyo and more were circumscribed or showed severe cellular reaction. Histologic characteristics associated with a more benign course were more frequent in Tokyo. However, the relationships between histologic type and survival were not consistent and the higher survival ratio of the Japanese patients could not be related to these characteristics.[152]

An epidemiologic study of the association of reproductive experience and cancer of the ovary was conducted on 399 patients and matched controls.[153] Women with ovarian cancer show the following characteristics: a lower mean number of pregnancies; a larger number of never-pregnant and women limited

to 1-2 conceptions; an increase in the relative risk as the total number of pregnancies decreases; a greater risk in ever-married never-pregnant than in never-married; a late age at first pregnancy; a greater interval between first marriage and first conception; a larger proportion of women who tried and failed to become pregnant at least once and an increase in the frequency of conceptions terminating as miscarriages. Both patients and controls tended to have fewer offspring than their mothers, but the difference was greater between patients and their mothers than between controls and their mothers. The results indicate that women who developed ovarian cancer had a gonadal status that predisposed them to both cancer of the ovary and low fertility.

An analysis[154] of data from the registry of clear-cell adenocarcinoma of the genital tract revealed a group of 16 patients with authenticated adenocarcinoma but with no exposure to stilbestrol or hormonal medication of any description during fetal life. This group is distinctly different from the group exposed to stilbestrol in utero since its ratio of primary cervix to vaginal tumors is 10:6, which is the reverse of the findings for the exposed group. These findings support the conclusions of the prestilbestrol literature that spontaneous primary vaginal adenocarcinoma in young women was almost unique, whereas such lesions in the cervix were only uncommon. Based on registry data, it has been estimated that in women exposed during fetal life there is about 30 percent risk of developing adenosis or other minor anomaly of the vagina or cervix and about 0.1 percent risk of developing malignant change.

The role of circumcision in the epidemiology of cancer of the cervix remains uncertain. A recent study[155] of 1,148 patients and controls matched by age and ethnic group failed to provide evidence that circumcision status was related to invasive carcinoma of the cervix, carcinoma in situ, or cervical dysplasia. These studies included actual examination of the circumcisional status of the marital partner of patients who were still married to first husbands.

Changing environment may be associated with the rise in mortality from tumors of the testis in Japan. The mortality rate has risen from 1.53/million/year in 1947-49 to 3.81 in 1966-70.[156] These rates are still low compared with those of U.S. whites. The rise in mortality in Japan was greatest in young adults and children, and the rates in the middle-aged and the elderly have also risen. Of the fatal testicular tumors in Japanese boys <15 years old, 86 percent occurred in the age range of 0-4, while in the U.S. in the same period of time 59 percent of the fatal cases in boys were also in the youngest age group. The increase in Japanese mortality rate could not be associated with particular years in the total period studied but rather, appeared to be related to increased lifetime risks with successively later years of birth.

Since there are marked international variations in mortality from gastric cancer, an epidemiologic analysis[157] of mortality from gastric cancer in Newfoundland has been undertaken in the search for environmental factors which may be causally related to this neoplasm. Most cancer deaths occurred in the eastern half of the province and the marked regional variations have

remained fairly constant for many years and do not appear to be dependent upon rural-urban factors or geographic isolation. The mortality rate appears to be decreasing slightly for both males and females and there has been less variation in mortality rates for both males and females since 1945. Mortality among males is twice that for females and there is a rapid increase in mortality rate for males at age 55 whereas for females the most rapid increase begins at age 60. This study reveals that the gastric cancer mortality of Newfoundland is high, by international comparisons, but is less than in the highest risk countries (Japan, Chili, Iceland).

Epidemiologic studies suggest a relationship between dietary factors and colon cancer. Recent data[158] support an etiologic function for animal fat and/or animal proteins which in turn exert significant effects on the composition of fecal constituents (bile acids and neutral sterols) including microflora. In order to study the effect of diet on intestinal microflora (determined by β-glucuronidase activity) a mixed Western, high meat, high fat diet was compared with a balanced nonmeat diet in 5 men and 1 woman (30 to 52 years of age). Enzyme activity was significantly higher in stools of subjects on a high meat diet as compared to the nonmeat regimen. Thus, intestinal flora of individuals on a high meat diet have a higher capacity to hydrolyze glucuronic acid conjugates than that of subjects on a meat-free diet. These reactions, including the release of carcinogenic or other toxic compounds, would be more likely to occur in the colonic lumen of populations on a mixed Western diet.

Recent studies[159] on dose-response, and temporal aspects of bronchogenic carcinoma in cigarette smokers support the claim that cigarette smoke acts as a carcinogen in humans. The relationship between cigarette dosage and age at which smoking began has been analyzed in a series of 486 men born between 1890 and 1899 who developed lung cancer between 1956 and 1965. These men smoked one-half pack per day or more. It was demonstrated that diagnosis was made at progressively younger ages as cigarette dosage increased and as the age at which the men began smoking decreased.

Temporal aspects of occupational bladder carcinogenesis were estimated in a group of men with bladder cancer whose occupations involved work in the rubber and leather industry or work with dyestuffs, paint and other organic chemicals.[160] Data from a case-control study demonstrated that the younger a man is when he is first employed in these hazardous occupations, the higher the cancer risk. Men first employed by age 25 have a risk 2.4 times that of men never so employed. There was virtually no excess risk if exposure began after the age of 25. Risk was greatest 41 to 50 years after beginning employment in these occupations (latent period). There was no change in risk with increasing duration of exposure; however, the increased duration of employment was associated with a shortened latent period.

Recent epidemiologic data[161] suggest that biological differences may influence susceptibility to the deleterious effects of ionizing radiation. A prospective study of a group of children exposed to intra-uterine x-rays and followed to determine subsequent mortality and development of leukemia shows that the total mortality experience of the white exposed group during

the first 10 years of life was almost twice that of the white control group. The death rate for leukemia was about 3 times higher for those exposed than for controls. In contrast, the black exposed and control groups had similar mortality experiences. No differences in mortality from malignant neoplasms (exclusive of leukemia) were found in the exposed and control groups. The mortality differential among whites remained when obstetrical and socio-economic factors were considered.

The link between histocompatibility antigen phenotypes and certain neoplasms has been investigated in 29 patients wtih melanoma.[162] Twenty normals and 29 patients with other types of metastatic cancer served as controls. Patients with melanoma (24 of 29 with metastases) had a significantly reduced frequency of HL-A5. There was a trend to a deficiency in the second sublocus but this was not statistically significant. It was suggested that HL-A5 deficiency may be associated with a tendency to develop metastatic disease. Typing data from families of 5 patients indicated that in one family the parents carried the HL-A5 antigen suggesting that the deficiency may be acquired by deletion or by genetic crossing over. Deletion of this sublocus may provide a genetic marker for melanoma.[163] In another study, patients with Hodgkin's disease were shown to have an increased frequency of the HL-A1 phenotype.[164]

Unusual clustering of Hodgkin's disease cases in a group of high school students and their associates has suggested horizontal transmission. To reanalyze the significance of these case linkages, two objective epidemiologic approaches (two-time period and index-secondary) were applied to test the hypothesis that in schools where a case had occurred in a teacher or student, more than the expected number of additional cases would appear among teachers and students who attended the same school.[165] Five of the eight secondary public schools with cases diagnosed during the 1960-64 time period had other cases diagnosed during the years 1965-69. None of the matched control schools had cases in the latter period. Schools with index cases had a significantly higher than expected number of secondary cases. These results support the concept of some form of transmission. This study suggests that an asymptomatic carrier state may play a major role in transmission and that age of cases and duration in a setting may be important. The study also suggests that for horizontal transmission to become apparent, an index patient might have to be in contact with large numbers of age-susceptible persons, some of whom might have additional predispositions to the disease.

A collaborative case-control study[166] of Hodgkin's disease was undertaken in 2 geographic areas to investigate factors determining host susceptibility. Patients with Hodgkin's disease and controls were matched by age, sex, race, and socioeconomic status. These data did not confirm tonsillectomy, appendectomy, or prior infectious mononucleosis, mumps or chicken pox as risk factors in Hodgkin's disease. There was a sixfold increase in prior history of dexedrine use in Hodgkin's disease patients over that for matched controls. This increased risk applied especially for the nodular sclerosis and mixed-cellularity types of Hodgkin's disease.

Seroepidemiology is one approach being utilized to study the association between cervical cancer and herpesvirus. In a case-control study in Yugoslavia the mean log titers of herpesvirus-2 (HSV-2) antibody were higher in cases than controls in each age group and among both Moslems and Non-Moslems.[167] In contrast, the HSV-1 antibody titers followed a random distribution in cases and controls. The ratio of HSV-2 to HSV-1 mean log titers was also higher in patients than in controls. Furthermore, HSV-2 antibody prevalence was greater among cases than among controls at all ages in each religious class. The risk of a prior HSV-2 infection was approximately twofold greater among women who had cervical cancer than among those who did not. These results support the hypothesis that herpesvirus is involved in the etiology of cervical cancer.

Seroepidemiology is also contributing to the study of the relationship of Epstein Barr virus (EBV) to human cancer and other diseases. Longitudinal serological studies[168] of African patients with Burkitt's lymphoma (BL) indicated that those patients who were brought to remission and showed no antibodies to the early antigen complex or lost them in time usually were found to become long-term survivors, with few, if any, early relapses; some have survived for 5-8 years. In contrast, those patients who retained or developed high anti-R and/or anti-D titers were prone to have multiple and eventually fatal relapses. All patients who died within 6 months had high anti-R titers. Since anti-R and anti-D are directed against intracellular antigens which are not expressed in vivo, the presence of these antibodies may reflect other adverse immunologic conditions. In another study[169] comparing 89 African and American patients with BL, it was demonstrated that nearly all African patients have high EBV antibody titers while one-third of the Americans had no detectable titers and when antibody was present, the titers were no higher than in controls. In this study, there was no correlation between antibody titer and the course of disease in either African or American patients. These results suggest either that African and American BL are different diseases or that EBV is unlikely to be the etiologic agent of BL in both Africa and America.

Antibodies to EBV were elevated in Hodgkin's disease patients with 3 histologic subtypes but were normal in patients with lymphocyte predominance subtype.[164] A case of sinus histiocytosis with massive lymphadenopathy was also associated with elevated EBV antibody titers.[170] Among 74 sarcoidosis patients, EBV, rubella and parainfluenza 3 antibodies were elevated.[171] In other studies no supporting evidence was found that EBV is a causative agent in systemic lupus erythematosus.[172]

Tumor-specific antibodies have been found in patients with transitional cell carcinoma of the urogenital tract.[173] The finding that the serum from one patient reacted not only against his own tumor but also against tumor from another patient suggests the presence of a common antigen(s) shared by at least some of these neoplasms. A newly identified RNA virus is associated with these tumors. Data suggest that antigens present on the tumor cell surface are related to those on transitional cell carcinoma-associated RNA virus. Further study will be needed to define the role of this RNA virus in human transitional cell carcinoma.

For the first time, antibodies reacting to simian virus 40 (SV40) T antigen have been detected in the sera of a renal transplant recipient and in the sera of 2 healthy children.[174] These sera had minimal or undetectable levels of SV40-neutralizing antibodies but high titers of antibodies to BK virus, an SV40-related papovavirus. Infection with BK virus has been shown to be common in childhood and is not known to be related to any illness. It was suggested that the observed reaction of human sera with SV40 T antigen is a result of a minor antigenic cross reactivity between T antigens of BK and SV40. Recent studies demonstrate that the antibodies found in sera of some breast cancer patients are specifically directed against mouse mammary tumor virus particles and these antibodies may fluctuate since one patient, positive on the first testing became negative 3 years later.[175]

Cattle herds provide an opportunity for large-scale seroepidemiological studies on the distribution of bovine C-type virus (BLV) and its relationship to bovine leukemia and persistent lymphocytosis (PL).[176] The majority (90 percent) of leukemic cattle and 80 to 100 percent of clinically normal cattle with PL in multiple-case herds had fluorescent antiviral antibodies. Antibodies were found in one-third of the cattle in single-case herds while antibodies were found in only 4 of 214 cattle from leukemia-free herds. The data suggest that BLV may be the etiologic agent of BL and perhaps of PL but that genetic and other viral factors may influence the disease. The observation that most cattle with leukemia and clinically normal animals in multiple-case herds possess naturally occurring antibodies to the intraspecies group-specific BLV antigen indicates that host interactions in bovine leukemia may be different from those in murine leukemia. The bovine system may offer a model for studying the natural history of human leukemia.

The distribution of incubation periods for several neoplasms was compared to the log normal distribution model reported for incubation times in infectious diseases.[177] The neoplasms selected for study included four different sets of radiogenic leukemias, thyroid cancers, thyroid adenomas, Wilms' tumor, bladder tumor and lung cancer following occupational exposure, pancytopenia attributed to chloramphenicol and Burkitt's lymphoma from four different countries. Most of the observations approximated a similar logarithmic normal distribution. The results for pancytopenia suggest that the log normal pattern may also apply to the distribution of incubation periods in other chronic diseases.

A comprehensive mathematical model has been constructed for the human granulopoietic system based on quantitative data involving all phases of granulocyte kinetics.[178] A detailed model was developed for an hour-by-hour description of the interrelationships of the kinetics of the various marrow cell types with the events in the peripheral blood and tissue spaces. The model was extended with a view to analyzing the effects of 5-fluorouracil on granulocyte kinetics so that it was possible to calculate a prediction of the hour-by-hour response of an ideal patient to the drug and to analyze the hour-by-hour response to the drug of the various marrow phases of granulocyte kinetics.

DIAGNOSIS

The search continues for biochemical markers specific for human cancer that may offer sensitive tests for early detection and diagnosis. While recent studies have demonstrated that the carcinoembryonic antigen (CEA) assay is not specific for colon cancer, quantitative and serial measurements give information useful for diagnosis, prognosis and possible monitoring of therapy.[179] It is also being evaluated for its usefulness in other tumor systems. Plasma CEA levels were elevated in approximately 50 percent of 341 patients with various gynecological cancers (cervix, vulva, ovary, and endometrium).[180] Positive CEA values were present in 84 percent of patients studied with recurrent tumors suggesting that measurement of CEA levels may prove of value in follow-up studies of these patients. Positive serum CEA levels in surgically treated breast cancer patients may detect metastases and give an indication for earlier chemotherapy.[181]

A membrane glycoprotein isolated from human breast carcinoma appears to be a CEA-like antigen that shares antigenic determinants with CEA.[182] This implies that the positive CEA radioimmunoassay observed among breast cancer patients might be due to the presence of circulating CEA-like, cross reacting antigen produced by breast cancer rather than that breast cancer is also producing CEA of colonic type. The results suggest that the antigen may also be present in benign breast lesions. A major effort is being made to purify and define the chemical composition of CEA[183] and various assays for CEA are being evaluated and compared[184] and modifications are being made in order to improve assay sensitivity.[185]

A novel low molecular weight glycoprotein, isolated from the urine of a patient with chronic myelocytic leukemia has been chemically defined and a specific antiserum has been produced and used to determine its incidence in various groups of patients.[186] This glycoprotein is a previously unrecognized member of normal plasma glycoproteins. Its plasma concentration frequently increases in patients with leukemia. It does not occur in normal urine but does occur in the urine of patients with leukemia, other disseminated neoplastic diseases, chronic inflammatory disease, tuberculosis or after surgery. This protein is not related to CEA.

Using a newly developed indigogenic, histochemical method, the presence of a unique isoenzyme 5'-nucleotide phosphodiesterase has been demonstrated in the sera of patients with primary and metastatic hepatoma.[187] The test appeared to be useful for distinguishing benign from malignant liver disease. False positive cases have been found in some noncancerous liver diseases but these can be distinguished from hepatoma by other liver function tests. Since no relationship was found between this isoenzyme and α-fetoprotein, a commonly accepted hepatoma marker, this isoenzyme may provide a useful alternate aid for diagnosing hepatoma, and a useful marker for liver damage.

Tissues which normally possess the A, B, or H isoantigens lose this characteristic when they become malignant. This phenomenon can be measured by an immunologic technique, the specific red cell adherence test. Absence of these antigens has been observed in carcinoma of the cervix, ovary, stomach,

lung, pancreas, and bladder. This test has now been used to study isoantigens in carcinoma of the fallopian tube.[188] The isoantigens A, B, and H, which are normally present in mucosa of this organ, were partially or completely lost in these carcinomas but were present in benign lesions. The loss of these antigens has been interpreted as evidence for immunologic dedifferentiation analogous to morphologic dedifferentiation of anaplasia. The sensitivity and specificity of this test may detect immunologic changes before histologic dedifferentiation is apparent.

Placental alkaline phosphatase, chorionic gonadotropin, and placental lactogen are all proteins normally synthesized only by the trophoblast but the ectopic synthesis of each of these polypeptides has been demonstrated in patients with non-trophoblastic tumors. Assays on sera of eight patients with non-trophoblastic neoplasms indicated that the presence of each peptide was independent of the others and that when more than one peptide was found, the concentration of each was independent of the others, suggesting that each of these proteins may be used as independent cancer markers.[189]

The endocrine and metabolic status of patients who develop endometrial carcinoma have been studied in an attempt to find possible etiologic clues. There was abnormal and excessive growth hormone secretion in 15 patients with endometrial carcinoma compared with controls matched by age, weight, and glucose metabolism. These concentrations were especially high at one-half, one, and two hours after insulin-induced hypoglycemia. The factors responsible for this excess HGH secretion need further study.[190]

A study was undertaken to determine whether a correlation exists between abnormal tryptophan metabolism and altered urinary steroid levels in a group of 25 patients with breast cancer.[191] Twelve of the 25 had abnormal excretions of metabolites of the kynurenine-niacin pathway and in these patients, the excretion of etiocholanolone was significantly lower than in controls or in patients with normal tryptophan metabolism. The possible addition of abnormal tryptophan metabolism to the present prognostic urinary steroid discriminants in breast cancer warrants further study.

Urinary excretion of nonesterified cholesterol (NEC) may be another marker related to cancer.[192] In a series of male patients elevated levels of NEC were observed in 29/32 patients with adenocarcinoma of the prostate, and in eight patients with choriocarcinoma, teratocarcinoma or embryonal cell carcinoma of the testes. Hyperexcretion of NEC was also present in patients with metastatic carcinoma of testes and prostate in which the primary tumor had been removed. Nineteen cases of testicular seminomas had normal excretion of NEC. NEC values appeared to correlate with the clinical course of the disease. The hyperexcretion of NEC in 25 percent of controls may be the result of unsuspected adenocarcinoma of the prostate. NEC excretion did not correlate with serum cholesterol, suggesting that urinary cholesterol is of endogenous origin. NEC hyperexcretion has previously been demonstrated in women with carcinomas of steroid-producing organs and their target organs.

Development continues on the automated cancer cell diagnosis system, TICAS. A decision procedure has been developed for testing of clinical cytology for

normalcy and adequacy.[193] In addition, it was determined that the classic Papanicolaou stain can be used in automated cell analytic procedures.[194] Feasibility studies suggest that computer discrimination of malignant from nonmalignant cells in pleural fluids may become a reality.[195] Data also indicate that histologic areas of lymph nodes representing thymus-dependent and bone marrow-dependent lymphocytes can be differentiated one from another and compared with the cortex and medullary areas of the thymus by these machine recognition processes.[196]

Automated differential leukocyte counts may offer a valuable means of monitoring therapy in patients with leukemia. In a preliminary study[197] differences in fluorescence emission of leukocytes supravitally stained with acridine orange could be measured by a flow-through cytofluorimeter and used to distinguish different classes of cells. Good correlation was found between the instrumental counts, manual counts and clinical status of the patients. Differential white cell count could be obtained with this method when the total white cell count was too low for reliable differential count by visual methods.

Tracheobronchial cytology may offer a new test for the detection and diagnosis of cancer. In a retrospective pilot study,[198] multinucleated tracheobronchial ciliated epithelial cells were found to be 2.08 times as numerous in smears from patients with a variety of malignancies as in patients without known malignancies matched by age, sex and smoking habit. In addition, a higher degree of multinucleation was found in patients with cancer. A prospective study is in progress to evaluate this phenomenon in an expanded series. An important aspect of this study is the search for occult cancer in patients with high percentages of tracheobronchial multinucleation in the control group.

Data from a comparative analysis of chromosomes with conventional diagnostic cytology in effusions from 58 cancer patients, show that chromosome analysis cannot substitute for the conventional approach, but appeared to add approximately 4 percent improvement in diagnostic accuracy.[199] Since most of these patients had been treated by x-ray and/or chemotherapy these chromosome analyses may be ambiguous. Chromosome analysis may be more valuable in untreated patients. A further potential advantage of this technique might be ready adaptation to automated diagnosis.

Sequential cytogenetic investigation of the chromosomal changes in adults with acute leukemia made it possible to categorize the patients into several profiles of aneuploidy.[200] Members of the C, D, E and G groups appeared to be more frequently affected. Of the 170 patients examined, 40.5 percent had chromosomal alterations during the acute phase of their disease. These abnormal cells disappeared from the bone marrow of patients entering remission while remaining in the nonresponders and relapse was accompanied or preceded by the return of the same abnormal clone. The median survival time found for the diploid patients was 49.9 weeks and for 69 aneuploid patients it was 32.8 weeks. Patients in some of these profiles appeared to have a very good prognosis.

A diagnostic method for sampling the endometrial cavity by negative pressure lavage has been evaluated in 31 patients with endometrial adenocarcinoma.[201] The Gravlee Jet Washer has shown an over-all diagnostic accuracy of 96.6 percent; there was one false negative and two unsatisfactory specimens.

A new instrument, the breast biopsy vacucylinder, has been designed, constructed, and tested on thirteen patients undergoing mastectomy.[202] Tissue specimens were obtained that were 3 or 4 mm in diameter. Preliminary experience with this technique has been without complication and specimens have provided more ample histologic preparations than those obtained by needle techniques.

Several studies are concerned with the problem of classification of cancers. An oncotaxonomy with symbols, based on linguistic analysis, has been developed to provide a basis for a unified classification of cancer.[203] The unified approach suggested by a symbolic language based upon a standardized oncologic anatomy would help to provide data that is measurable, verifiable by others, and codifiable and would allow a better means of reaching treatment decisions. A simplified classification of the normal and malignant histiocytoses based on cellular differentiation has been proposed.[204] By combining functional, morphologic and biochemical aspects of histiocyte differentiation it should be possible to classify these disorders in a patient in a manner that reflects the biologic properties of the proliferating cell.

A definite diagnostic decision between malignant nodular (follicular) lymphomas and benign lymphoid follicular hyperplasias is sometimes difficult and there can be a risk of misinterpretation by histologic criteria. An immunofluorescent technique using fluorescein-labeled antibodies against human immunoglobulins was used to study frozen tissue sections of lymph nodes and spleens of 12 patients with lymphoma.[205] Immunoglobulins detected by this method in the neoplastic nodules were minimal or absent and in no instance could the characteristic network pattern of immunoglobulin distribution, which is readily demonstrated in active germinal centers, be observed in these nodules; thus, the preservation of the follicular network of immunoglobulins indicates functional intact germinal centers. The detection, by this simple technique, of large amounts of immunoglobulin distributed in such a distinctive pattern may be a useful tool for the recognition of severe benign follicular hyperplasia.

The development of radiopharmaceuticals suitable for photoscanning various organ sites has led to useful diagnostic agents for detecting cancer. ^{131}I-19-iodocholesterol adrenal scans have successfully detected pheochromocytoma preoperatively in six patients.[206] Although this radiolabeled cholesterol does not localize in adrenal medullary tissue, it is able to detect distortion of the cortex by tumor tissue. Investigations continue on the development of a gamma-labeled dopamine derivative to be used in the diagnosis of adrenal medullary disease. 19-iodo-β-sitosterol-^{125}I has been synthesized and in assays in rats exhibited selective localization in adrenal cortical tissue.[207] Esters of 19-radioiodinated cholesterol were synthesized and were shown to concentrate in rat adrenals only after oral administration.

The apparent ability of these radioiodinated sterol esters to serve as substrates for pancreatic cholesterol esterase suggests their possible use in the diagnosis of pancreatic insufficiency.[208] Bretylium compounds are also being synthesized and assayed in the rat as potential myocardial imaging agents.[209]

Gallium-67 is undergoing intensive study as an aid in tumor diagnosis. ^{67}Ga concentrates in a variety of tumors and normal tissues, and current studies are investigating the factors involved in its affinity for certain cell types. In 2 patients with renal cell carcinoma, studied by means of ^{67}Ga scanning, there was excellent uptake of the radionuclide in the primary tumor as well as in the metastatic sites.[210] In soft tissue tumors of rat and mouse, ^{67}Ga appeared to be associated with lysosomal granules.[211] Studies indicate that a large portion of the extractable macromolecular substance(s) that binds ^{67}Ga in tumor and normal liver tissue is sensitive to both heat and alkalinity. There appeared to be a quantitative if not qualitative difference between tumor and normal tissues with respect to the macromolecular species that binds ^{67}Ga.[212] Purification and determination of the exact nature of this macromolecular species will be required to determine whether this represents a true difference between malignant and normal cells. In other studies, carrier-free scandium-47 has been isolated from calcium-47, and its utility as a tumor or bone marrow scanning agent was suggested by its selective distribution in tumor-bearing mice.[213]

Research continues on the possible use of NMR for the diagnosis of cancer. It has been shown by several investigators that the nuclear magnetic relaxation times [spin-lattice (T_1) and spin-spin (T_2)] of tissue water protons differ in normal as compared with malignant tissues. Current investigations are concerned with the mechanisms that could account for these observations. When the T_1 nuclear magnetic relaxation times and water contents of various normal and malignant mouse tissues were correlated, increasing T_1 was associated with higher water contents of tissue.[214] When the water contents of liver and kidney tissues were increased artificially, high water content was again associated with high T_1. Solutions of bovine serum albumin of different concentrations showed increased T_1 of water associated with decreased protein concentrations. The transplantable mouse tumors did have more water than normal tissues and thus longer relaxation times; however, elevated relaxation times are also found in other nonmalignant conditions. Further study will be needed to determine the applicability of NMR for cancer detection.

PHARMACOLOGY

The alkaloid thalicarpine, which has the aporphine benzylisoquinoline structure has been reported to effect a significant inhibition against the Walker 256 intramuscular carcinosarcoma in rats over a wide dosage range. In anticipation of potential needs for the alkaloid in a quantity far exceeding that readily available from natural sources, the practical total synthesis of thalicarpine was achieved. A current report describes an approach which utilizes readily available starting materials and an activated aryl

halide as a precursor in an efficient diaryl ether total synthesis.[215] The alkaloid has undergone extensive preclinical toxicological studies, and is now in clinical trial.

In continuing search for tumor inhibitors of plant origin, alcoholic extract of Eupatorium cuneifolium Willd, a plant of the Compositae found in Florida, showed significant inhibitory activity against KB cells from human carcinoma of the nasopharynx. A preliminary communication[216] described the isolation of the novel antileukemic germacranolide eupanucin, plus two other cytotoxic germacranolides, eupacunoxin and eupatocunin. A current report[217] presents in detail the stereochemistry of these germacranolide lactones and the isomeric germacranolides, eupatocunoxin and eupacunolin. These sesquiterpene lactones appear to be the first recognized naturally occurring germacranolide cis,cis-dienes. The most abundant of these lactones, eupacunin was tested in vivo and was found to show a significant inhibitory activity against the P-388 leukemia in mice and the Walker 256 carcinosarcoma in rats.

A recent report[218] describes the synthesis and antitumor action of a bidentate arylamine congener of cis-diamminedichloroplatinum II (cis-Pt (II)), dichloro (4,5-dimethyl-o-phenylenediamine-N,N') platinum (II) (DDPP) which was synthesized by the reaction of 4,5-dimethyl-o-phenyldiamine with potassium tetrachloroplatinate. DDPP extended up to 176 percent the survival times of BALB/c mice bearing the Ehrlich ascites tumor, and up to 74 percent the survival times of BDF_1 mice bearing the L1210 leukemia. In studies using Ehrlich ascites tumor cells in vitro, DDPP was found to be a potent inhibitor of synthesis of DNA, RNA, and protein. The concentrations which effected 50 percent inhibition following 1.5 hr of incubation with the compound were $3x10^{-5}$ to $5x10^{-5}$M. The inhibition of nucleic acid and protein synthesis in vitro, once established, was not ameliorated by washing the cells with fresh medium devoid of DDPP. The rate of loss of in vitro cell viability with DDPP was considerably greater than with an equivalent molar concentration of cis-Pt (II).

Cis-Pt (II) has shown effectiveness against some human cancers but dose-limiting renal toxicity occurred in about 25 percent of the patients given 50 mg/sq m, and in 61 percent of the patients given 100 mg/sq m in a single dose. A Phase II study was undertaken to compare the therapeutic activity of cis-Pt (II) at a single dose level of 50 mg/sq m with that achieved by 5-fluorouracil (5-FU), administered in a 5 day course either by rapid i.v. injection at a dose level of 13.5 mg/kg/day, or orally at a dosage of 20 mg/kg/day.[219] Twenty-nine patients who had histologic confirmation of unresectable cancer of the colon or rectum and had not received previous chemotherapy were randomly assigned to either cis-Pt (II) or 5-FU therapy. In addition, 12 patients who had previous chemotherapy, were entered directly into the study with cis-Pt (II). Among the 32 patients treated with cis-Pt (II), 25 showed definite tumor progression at 5 weeks and were not re-treated. Five remained objectively stable and received a second course of cis-Pt (II) therapy at 5 weeks. Four of these patients showed tumor progression at 10 weeks, and one has remained stable at 15 weeks after three courses of cis-Pt (II) therapy. Two patients showed mixed responses.

Those patients who showed tumor progression while receiving cis-Pt (II) were crossed over to 5-FU, and those receiving 5-FU were crossed over to cis-Pt (II). Four of 23 such patients (17.5 percent) who were treated with 5-FU showed an objective (>50 percent) response which was maintained for 10 weeks after the start of therapy. An additional 3 patients showed objective responses at 5 weeks but tumor progression at 10 weeks. No objective responses were noted among the 19 patients treated with cis-Pt (II). At the dose level used in this study, cis-Pt (II) appears to have little value in the therapy of colorectal adenocarcinoma, and it is significantly inferior to 5-FU. The low hematologic toxicity of cis-Pt (II) may allow it to be used in combination with other drugs which do not depress bone marrow function.

L-asparagine amidohydrolase (EC 3.5.1.1) apparently inhibits protein synthesis in those tumor cells which lack asparagine synthetase. The possibility that this mechanism might be enhanced by dietary means was demonstrated in C3H/He mice bearing the L-asparaginase-sensitive 6C3HED tumor.[220] On the other hand, the antitumor effect of L-asparaginase can be offset by the administration of excess asparagine or glycine to the tumor-bearing host. This is believed to be related to the in vitro observation that glycine inhibits asparagine amidohydrolase in guinea pig liver. In both systems the competitive inhibition of glycine is specific, not a property of amino acids in general.[221] The observed immunosuppressive effects of L-asparaginase on cellular and humoral responses may be explainable by a study of the effect of L-asparaginase on the surface of lymphocytes. Electron micrographs show that although L-asparaginase is unable to cleave off concanavalin A surface receptors, it does cause a significant clustering effect on the topographic distribution of such membrane concanavalin A sites.[222] Another study to elucidate the basis for loss of therapeutic effectiveness in sensitized animals, and which may relate to clinical reports of the antigenicity of L-asparaginase, suggests that these effects may be attributed to reduction of catalytic activity by antibody and, more importantly, to extremely rapid clearance from the sensitized tumor-bearing host's circulation.[223]

A new enzyme termed GA:1.2, possessing approximately equal amounts of glutaminase and asparaginase activity, has antitumor activities against cancers other than leukemia, thus enlarging the potential for cancer therapy by amino acid deprivation.[224] When tested against the asparagine-dependent EARAD-1 mouse leukemia in the presence of the lactate dehydrogenase-elevating virus (LDH-virus), GA:1.2 induced marked tumor regression. At doses of 150 IU/kg and higher, both plasma glutamine and asparagine were depleted to undetectable levels when the virus was present but not in its absence; tumor regression was correlated with asparagine and glutamine depletion in the plasma. The plasma half-life of GA:1.2 is 1 to 2 hours in normal mice, but was increased to 12 to 18 hrs when mice were infected with the LDH-virus. Thus, the presence of the LDH-virus in the host is necessary for expression of the therapeutic capabilities of the enzyme preparation on this and presumably other mouse tumor systems.

Guanazole (3,5-diamino-1,2,4-triazole, NSC-1895) is a new antitumor agent which appears to act by inhibiting ribonucleotide reductase and thus specifically blocking DNA synthesis. In a Phase II study,[225] 24 leukemic patients were treated by continuous intravenous infusion, 7.5 g/sq m/day for 5 days. The 5-day courses were repeated every 14 days. There were 4 complete remissions, 2 partial remissions, and 4 hematologic responses. The complete remissions in 3 patients with acute myelogenous leukemia lasted 49 days in one patient, and the two other patients were still in complete remission at 45 and 230 days after treatment. The one patient with chronic myelogenous leukemic blast transformation achieved a complete remission that lasted 99 days. Myelosuppression was the major toxic effect.

In another clinical trial[226] of guanazole given to 27 patients with advanced neoplastic diseases, mostly acute leukemia, the drug was given in 5-day courses; dosages ranged from 5 to 40 g/sq m/day administered by continuous infusion. Complete remission was achieved in two of 14 patients with previously treated acute myelocytic leukemia, and an M-1 marrow occurred in one of four patients with advanced acute lymphocytic leukemia at a dose of 25g/sq m/day x 5 after no response to 20g/sq m/day x 5. The beneficial responses to guanazole appear to be dose-related with a narrow therapeutic index. No significant toxicity except fever was seen at any dose of guanazole below 10 g/sq m/day. Myelosuppression was the most significant dose-related toxic effect, but mucositis, rash, and alkalosis were also observed.

CHEMOTHERAPY

Androgenic therapy for advanced breast cancer in women has been used for several decades and produces objective regressions but the regression rates for metastatic lesions continue to range between 10 and 20 percent. A recent report[227] details a Phase II double-blind, randomized study of two androgenic compounds: calusterone (7β,17α-dimethyltestosterone) (NSC-88536) and Δ^1-testololactone (17α-oxa-D-homoandrosta-1,4-diene-3,17-dione) (NSC-23759). Objective remission of advanced breast cancer was achieved in 28 percent of the 109 patients receiving 200 mg calusterone orally daily, and in 18 percent of the 115 women receiving Δ^1-testololactone at an oral dosage of 1 gram daily. The mean remission duration of 11.9 weeks and one-year survival rate of 59 percent for the patients on calusterone therapy were comparable to remission duration of 12 weeks and one-year survival rate of 59 percent for those on Δ^1-testololactone.

The efficacy of calusterone was also evaluated in another group of 117 women with progressing advanced breast cancer. In 41, calusterone was the first treatment; in 49 it was given as secondary steroidal therapy; and in 27 it followed cytotoxic chemotherapy. Calusterone produced objective regressions lasting an average of 9.8 months in 51 percent of patients in whom it was primary or secondary hormonal therapy, but in only 19 percent of patients with previous cytotoxic chemotherapy. In the last group, regressions lasted only 6 months. Calusterone was equally effective in women with or without visceral metastases, in all age groups, and in castration failures.

Calusterone is a weak androgen, and, like other 17α-alkylated steroids, produced nausea in 28 percent of patients and bromsulphalein retention almost uniformly. There was no cholestatic jaundice or hepatocellular damage. Unlike other androgens, calusterone causes no rise in hematocrit, but a striking increase in the platelet count and a slight rise in the WBC count. By the rigorous criteria of the Cooperative Breast Cancer Group, calusterone is the most effective antitumor steroid thus far identified in a statistically adequate sample.

In a double-blind study,[228] calusterone produced regressions in 52 percent of 23 patients as compared with 32 percent of 25 patients treated with Δ^1-testololactone. In the crossover, patients who had not responded to calusterone also failed to respond to Δ^1-testololactone, while 33 percent of Δ^1-testololactone failures or responders obtained regression on calusterone therapy.

A prospective randomized dose response evaluation of Δ^1-testololactone in women with advanced breast cancer showed a significant difference in the remission rates between the lowest and the two higher dose levels: 200 mg, 5/118 (4.2 percent); 1000 mg, 18/123 (14.6 percent); and 2000 mg, 15/121 (12.4 percent). The incidence of severe side effects was lowest with the 1000 mg dose. Δ^1-Testololactone is thus effective for the palliative primary treatment of women with advanced breast cancer. It has the unique advantage of having no significant metabolic, masculinizing, or feminizing hormonal activity. An increase to 2000 mg per day should be considered in a patient with visceral metastases who fails to respond favorably to the 1000 mg dosage.[229]

Adriamycin (14-hydroxydaunomycin) is an anthracycline antibiotic found in the fermentation products of Streptomyces peucetius that has been found to be active against various animal tumors, and to have a higher therapeutic index than daunomycin. In a Phase II study of adriamycin, objective remissions were seen in 118 (25 percent) of 472 patients, with best results noted in lymphomas (21/48), sarcomas (21/64) and carcinoma of the breast (16/50). Eighty-nine percent of remissions occurred within three courses. Hematopoietic toxic effects were seen in 73 percent of patients; nausea, vomiting and/or stomatitis were observed in 43 percent. Changes in electrocardiograms were seen in 42/472 patients after cumulative doses of adriamycin ranging from 45 mg/sq m to 600+ mg/sq m. Irreversible congestive heart failure occurred in two patients after cumulative doses of 555 and 825 mg/sq m, respectively. It was concluded that adriamycin is an active agent, most remissions occur promptly, but significant cardiotoxic reactions appear to be cumulative.[230]

Adriamycin, alone or in combination with other drugs, was evaluated in the treatment of advanced malignant lymphomas.[231] Eighteen patients who had become refractory to extensive combination chemotherapy received adriamycin alone at doses of 60 to 75 mg/sq m, i.v. every 3 weeks. Five achieved complete remissions, and four had partial remissions that continued for a median duration of more than 7 months, with five patients still in remission at the time of the report. A second regimen included 16 patients who had become

refractory to initial combination chemotherapy or who had had extensive bone marrow involvement including frank leukemia. These patients received adriamycin in combination with cytosine arabinoside, vincristine and prednisone. Four complete and four partial remissions were observed for a median duration of 2.5 months with four patients still responding. In a third regimen, 9 patients with minimal or no previous chemotherapy received adriamycin combined with cyclophosphamide, vincristine, and prednisone. All nine patients responded; seven complete and two partial remissions were observed for a median duration of more than 8 months, with 6 patients still in complete remission. For the 15 patients still in complete remission on all three regimens, the median duration of response at the time of reporting was more than 11 months. In the patients with non-Hodgkin's lymphoma, the response rate was similar to the different histological subtypes. Major side effects include nausea, alopecia, and myelosuppression, with the latter side effect most pronounced in the combination with cytosine arabinoside. The response rate with the combination of adriamycin, cyclophosphamide, vincristine and prednisone is particularly encouraging in non-Hodgkin's lymphoma.

Adriamycin was given to 234 patients with leukemia and other types of neoplastic diseases at the Memorial Sloan Kettering Cancer Center from June 1969 through August 1971.[232] In children with previously treated acute leukemia, adriamycin produced complete remissions (12 percent) and good partial remissions (26 percent). Tumor regressions were seen in 60 percent of all patients with solid tumors. These included lymphoma, embryonal rhabdomyosarcoma, neuroblastoma, Ewing's sarcoma, Wilms' tumor, ovarian tumor, hepatoma, embryonal carcinoma, malignant teratoma, and retinoblastoma. The therapeutic responses in adults have been less consistent and are confined to lymphomas and soft tissue sarcomas. The dose in children was 0.5 mg/kg daily to a total of 2-4 mg/kg per course. In adults, the single dose was 0.4 mg/kg to a total of about 2.5 mg/kg. in 10 days. Adriamycin at half the dose of daunomycin produced comparable toxicity, except for earlier and more frequent oral ulcers and alopecia. Varying degrees of transient electrocardiographic changes have been seen in children. Cardiac failure and death occurred in one treated child who had recurrent pulmonary metastases.

The nitrosoureas are a unique group of antineoplastic agents: they are lipid-soluble, cross the blood-brain barrier, are distributed widely to tissues, and have an extremely short plasma half-life. 1-(2-Chloroethyl)-3-cyclohexl-1-nitrosourea (CCNU) (NSC-79037) is of special interest because the cyclohexyl group enhances lipid solubility. CCNU was used to treat 141 evaluable patients with a variety of advanced solid tumors, 56 percent of whom had not received prior chemotherapy.[233] Patients with an adequate bone marrow reserve received 130 mg/sq m as the initial dose, and those with an impaired reserve received 100 mg/sq m. Therapy was repeated every six weeks and the dosage modified according to the hematologic toxicity. Responses greater than 50 percent in tumor size were seen in 26 patients; stable disease or reduction in tumor size less than 50 percent was noted in 21 patients. The well-differentiated adenocarcinomas and squamous cell tumors appeared to be the least sensitive to CCNU. Bone marrow depression was the major dose-limiting factor. Without adequate antiemetic therapy, all patients would have had nausea and vomiting. Hepatocellular toxicity was noted in two patients.

5-Azacytidine (NSC-102816) is a pyrimidine nucleotide analog produced by a species of Streptoverticillium. The drug is converted to a nucleotide by uridine kinase and is subsequently phosphorylated and incorporated into RNA; this modified structure inhibits protein synthesis. The diphosphate is a substrate of the nucleotide reductase and, subsequently, DNA pronase; thus, the drug is also incorporated into DNA. Previous studies have shown that this drug is active against a number of solid tumors and acute leukemia. 5-Azacytidine was administered to 28 patients with acute leukemia, and to four patients with chronic myelogenous leukemia in blast cell crisis, all but one of whom had received extensive prior therapy.[234] Four complete and four partial remissions were achieved in 29 evaluable patients. The median duration of complete remission was 19 weeks. Nausea, vomiting and diarrhea were common. Hypotension following administration of the drug occurred in four patients, and in one case this was fatal.

Intra-arterial infusion of cancer chemotherapeutic agents delivers a high concentration of the particular agent to localized malignant disease which has its blood supply entirely perfused by an accessible artery. In addition to the higher concentration of drug delivered directly to the neoplastic tissue, there is also less systemic toxicity because a significant amount of the agent is removed by the prior circulation through the capillary bed of the target region, thus keeping the concentration in the larger vascular compartment beyond the tumor area at levels low enough usually to prevent severe systemic toxicity. Seventeen patients with advanced regional malignant melanoma were treated intra-arterially with 5-(3,3-dimethyl-1-triazeno) imidazole-4-carboxamide (DTIC).[235] The usual dosage was 250 mg/sq m for 5 consecutive days, with 16 days off therapy and the next course begun on day 22. The range of dosage was 50 to 400 mg/sq m infused over 8 to 24 hours each day in 5 percent dextrose in one-fourth normal saline with 2,000 units of heparin. There were 6 partial (greater than 50 percent reduction) responders and one complete remission for an overall response rate of 41 percent. The local control of melanoma achieved by this modality of treatment was impressive, and toxicity was less than that which is usually seen with systemic DTIC chemotherapy, suggesting that intra-arterial therapy is preferable to systemic therapy for isolated inoperable melanoma.

A total of 392 evaluable patients with all types of metastatic cancer were entered on a cooperative study of vincristine therapy.[236] Tumor regressions occurred in a substantial proportion of patients with the following tumor types; advanced lymphosarcoma 13/26, reticulum cell sarcoma 9/24, Hodgkin's disease 20/41, breast cancer 15/62, bladder cancer 3/10 and carcinomas of unknown primary origin 5/15. Responses occurred about as frequently at 25 µg/kg/week as at higher doses. Toxicity varied from acute and self-limited to delayed and irreversible and was most frequently of dose-related sensory, motor, or autonomic neurological type. Paresthesias, described as numbness, tingling, burning, or prickling appeared in over half of the patients and thus can be considered characteristic of vincristine administration. The mechanisms of vincristine neurotoxicity and antitumor activity are unknown.

Cis-diamminedichloroplatinum (cis-Pt(II))was used in the treatment of 26 patients with advanced unresectable cancer who had failed to respond to

conventional therapy.[237] The scheduled cis-Pt(II)was dissolved in physiologic saline and administered by i.v. infusion over a 5-10 min. period. In the first patients treated, the initial dose was 0.03 mg/kg/day, and dosage was increased by graduated increments at 4-day intervals until toxic side effects occurred. Subsequent patients received various daily doses between 0.25 and 2.0 mg/kg for total course doses of 1.25-7.0 mg/kg. Of the 21 evaluable patients, 12 received a single course of therapy, five received two separate courses, three received three courses, and one patient received four courses. Thus, a total of 35 individual courses of therapy were evaluable for toxicology study. Seven of 21 evaluable patients experienced some therapeutic benefit. All except one of the responses occurred in patients with epidermoid carcinomas of the head and neck region, and all responses were of brief duration. Although the responses were limited in degree and duration, they demonstrated the definite antitumor potential of cis-Pt(II). Limiting toxic effects appear to be cumulative renal function impairment and apparently irreversible, high-frequency hearing loss. Toxicity is only roughly dose-related. No idiosyncratic reactions were observed.

COMBINATION THERAPY

Preliminary results of comparative clinical studies involving over 500 patients with Stage III-B and IV Hodgkin's disease indicate that the two 4-drug, MOPP and BOPP, regimens which for previously untreated patients give response rates of 88 percent and 90 percent, respectively, are more effective, though more toxic than either of two 3-drug regimens that yield about 70 percent response rates. The 3-drug regimens have therefore been terminated, and present efforts are directed toward slight modifications of the MOPP and BOPP regimens to achieve less toxicity without decreasing the tumor response. The MOPP regimen combines mechlorethamine hydrochloride, vincristine sulfate, procarbazine hydrochloride and prednisone. The BOPP regimen is similar to MOPP except that mechlorethamine hydrochloride is replaced by 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU). For patients treated with the MOPP program for remission induction, the maintenance treatment with vinblastine or chlorambucil and monthly vincristine and prednisone, seems superior to chlorambucil alone.[238]

In order to further explore the therapeutic potential of the MOPP regimen for patients with advanced Hodgkin's disease, 164 patients with Stage III or IV Hodgkin's disease were treated monthly with this regimen.[239] An overall complete remission rate of 65 percent was achieved. Patients with little or no previous therapy achieved a 79 percent complete remission rate. This study demonstrated that the MOPP regimen can be used not only in previously untreated Hodgkin's patients but also in patients given previous major chemotherapy or radiotherapy. Furthermore, the value of the MOPP regimen for maintenance treatment is indicated by the fact that the extrapolated duration of remission was approximately doubled by MOPP maintenance courses given every two months. During the one- to two-year follow-up period, only 8 of 35 maintained patients had a relapse, whereas 14 of 33 unmaintained patients relapsed.

A new program for treating advanced Hodgkin's disease employs five drugs and low-dose radiotherapy.[240] Three cycles of mechlorethamine HCl, vincristine, procarbazine, vinblastine and prednisone are given over six months, followed by 1,500-2,500 rads to all diseased areas. Following radiotherapy, two additional cycles of chemotherapy are given. Of 34 patients in this program, 25 have completed three cycles (six months) of drug treatment plus irradiation. Twenty of the 25 are in complete remission, continually free of disease, with a median remission duration of over 12 months. The principal toxic manifestation of this program is bone marrow depression. The clinical data seem to support the initial assumptions that irradiation is necessary in both stages III-B and IV, and the optimum dose is 1,500 to 2,500 rads. The experience of the Southwest Cancer Chemotherapy Group is of considerable interest in that they reported[241] that patients who relapsed following complete remission induced by MOPP did so at the sites of bulk disease. They suggested "local radiotherapy to the sites of largest disease will be essential to improve the effectiveness of MOPP therapy regimen."

Forty-nine children and adolescents with previously untreated Hodgkin's disease were given 12 weeks or more of intense combination chemotherapy with simultaneous radiotherapy at a tumor dose of 3500 to 4000 rads. The intensity and duration of the chemotherapy depended upon the disease stage.[242] For stages II-IV chemotherapy was continued for 1 to 2 years. Forty-seven of the 49 patients responded to therapy with complete remissions; two patients had no response. Of the 47 patients responding, 40 have been in continuous complete remission for periods of 6 to 58 months, four have relapsed, and three have died in remission. Twenty-six patients have been off all therapy for periods of 4 to 54 months without evidence of active disease. In most instances complications of therapy including drug toxicity, infections and nutritional disturbances have been reversible by modification of chemotherapy and judicious supportive care. Presently the effectiveness of this therapeutic regimen in producing a high remission rate without prohibitive toxicity but with prolonged complete remission in childhood and adolescent Hodgkin's disease is not defined. Whether simultaneous treatment is more effective than either modality used singly will be ascertained by a comparative study now in progress.

In an attempt to improve the 41 percent incidence of remission induction of childhood acute lymphocytic leukemia previously achieved by the administration of asparaginase alone, a clinical study was undertaken with 46 children in relapse with acute lymphocytic leukemia and resistant to conventional therapy.[243] One group of 24 children received 10,000 I.U. of asparaginase/sq m and 300 mg of cytosine arabinoside/sq m on days 1 and 8. The second group of 22 children received identical doses of these two agents plus 15 g/sq m of 6-azauridine on days 1 and 8. Complete remission (median duration 48 days) was induced in 15 of the first group and in 13 of the second group (median duration 52 days). Compared with asparaginase alone, asparaginase combined with cytosine arabinoside increased the incidence, but not the duration, of remission induction. No significant advantage resulted from addition of 6-azauridine to the asparaginase-cytosine arabinoside combination.

Progress in the treatment of adult acute leukemia has failed to match the succession of advances that have been achieved in the past decade in the treatment of childhood acute leukemia. A combination chemotherapeutic program for the treatment of adults with acute myelogenous leukemia (AML) and acute lymphocytic leukemia (ALL) was devised on the basis of theoretical considerations and experimental evidence that a combination of cell cycle-nonspecific and cell cycle-specific agents should be used for induction therapy.[244] The drugs, cyclophosphamide, cytosine arabinoside, and methotrexate (CAM), were given as weekly pulses until marrow hypoplasia or remission was attained. For remission induction therapy, patients with AML were treated with CAM; patients with ALL received CAM with leucovorin rescue (CAM-LO). Complete marrow remissions were achieved in 7 of 14 patients with AML, and in 5 of 8 patients with ALL. Toxicity from the combination was tolerable, although occasionally severe. This induction program compares favorably with other induction regimens in adult acute leukemia, but the short remissions underscore the need for the design of new approaches to improve maintenance therapy. These considerations have led to the design of a cooperative study to compare CAM with thioguanine and cytosine arabinoside for remission induction, and to use the two regimens sequentially for remission maintenance.

A combination of 6-mercaptopurine (Purinethol), vincristine (Oncovin), methotrexate and prednisone, POMP, induced complete remission in 40 percent of 89 adults with acute myelocytic or acute lymphocytic leukemia, and improved their survival to a median of 13 months as compared with a median survival for the entire group of 7 months. Age and previous therapy were major factors influencing the response of the patients in this study.[245] Side effects other than myelosuppression resulted in liver function abnormalities, minor gastrointestinal intolerance, and paresthesias. POMP chemotherapy is effective remission induction and maintanance therapy for adults under the age of 50 with acute leukemia, who have not had prior chemotherapy.

Between 1966 and 1968, 130 previously untreated patients with acute lymphocytic leukemia (100 under 20 years of age, and 30 over) were treated with combination chemotherapy. The specific aims in this study were to increase the number of complete remissions (CRs), particularly in adults; to lengthen the duration of the CRs; to prevent meningeal leukemia with intrathecal doses of methotrexate; and to increase the number of long-term survivors?[246]

The overall results of induction therapy with prednisone and vincristine included 117 CRs of the total 130 patients. The remissions in the children's group, 95 CRs/100 patients was significantly better than the 22 CRs/30 patients for the adult group. The remission rate for females, 40 CRs/40 patients, exceeded that for males, 77 CRs/90 patients, and may be linked to better resistance to infections in females. Further analysis shows that complications occurring during induction therapy are more often the cause of death (9/130) than is resistance to treatment (4/130). Maintenance therapy consisted of 6-mercaptopurine plus methotrexate. The median duration of hematological remission was 34 months in the younger group and 11 months in the older group. In patients between 15 and 20 years of age, the prognosis was the

same as in the older patients. The prognosis varied according to age; the initial WBC count; and the number of doses required to achieve initial CR.

Meningeal leukemia occurred in 33 percent of the cases in spite of preventive intrathecal doses of methotrexate, and in one-half of these cases meningeal leukemia occurred during the first year of the disease. However, 20 percent of these early meningeal leukemia cases showed no hematological relapse 5 years later, and 48 percent of the younger group who attained complete remission survived for more than 4 years, and 30 percent for 5 years.

Twenty women and 25 men (aged 15-80, median 43) with acute non-lymphocytic leukemia were treated with daunorubicin and prednisone.[247] Seventeen patients had received prior treatment, generally with prednisone, prednisone and 6-mercaptopurine,ara-C, or vincristine, prednisone, amethopterin and 6-mercaptopurine (VAMP). Six of these 17 patients had prior partial or complete remissions. Twelve complete and four partial remissions were obtained. The most important factors influencing remission induction were age and sex, and pretreatment absolute blast count (ABC). All 14 men over the age of 40 failed to respond, while 50 percent of all other patients responded. Patients with low ABC were more likely to respond. A review of the literature reveals a similar overall complete remission rate of 26 percent using daunomycin alone; thus, adding prednisone to daunorubicin does not appear to increase the percentage of complete remissions. Hydroxyurea, 80 mg/kg orally as a single dose twice weekly as long as WBC count was greater than 2,000/mm^3, resulted in longer remissions than have been previously reported following daunorubicin induction.

To evaluate the possible synergistic antitumor activity of chlorambucil and prednisone, and to determine whether prednisone might counter-balance the myelosupressive effect of chlorambucil, a prospective double-blind study was undertaken with 26 patients with chronic lymphocytic leukemia.[248] Eleven patients were treated with chlorambucil alone, and 15 received combined chlorambucil-prednisone therapy. Complete remissions were observed in 3, and partial remissions in 10 of the patients in the latter group. Only one patient treated with chlorambucil alone obtained complete remission and four showed partial remissions. Hematologic toxicity was less frequent in the combination therapy group. Two-year survival was 93 percent for the combination group, and 54 percent for the chlorambucil-treated group. This difference was not statistically significant, but the overall experimental data indicate that chlorambucil-prednisone combinational therapy is superior to chlorambucil alone in the treatment of chronic lymphocytic leukemia.

Rhabdomyosarcoma is among the ten most common cancers occurring in children. The embryonal variant of rhabdomyosarcoma is the type most often encountered and the pelvic portion of the urogenital tract is a frequent location for this tumor. It is highly malignant with a tendency toward early infiltration of adjacent structures, and metastases to regional lymph nodes and to distant organs. A recent review of 27 children under 15 years of age with embryonal rhabdomyosarcoma of the urogenital tract who were treated between 1960 and 1971 reveals that early stage of the disease at the time of diagnosis,

and multidisciplinary therapy (surgery, radiotherapy, and multiple drug therapy) offer the best chance of cure and survival.[249] Seventeen of the 27 children were still living, having remained free of the tumor for intervals ranging from 18 months to 10 years.

Combined systematic therapy offers the best chance for cure of children with rhabdomyosarcoma originating in the head and neck. Recommended therapy includes: 1. local excision or biopsy; 2. radiation therapy with doses of 6,000 rads/6 weeks; and 3. multi-drug chemotherapy, i.e., vincristine sulfate, actinomycin D, and cyclophosphamide. Of 19 patients, so treated, 14 are living with no evidence of disease between 2 and 7 years, with a median of 2 years 8 months. Local control of disease was achieved in 89 percent. Five patients died. Severe ocular, dental, soft tissue and possibly bone complications may be encountered. Late recurrences or complications require follow-up.[250]

The heterogeneity of head and neck tumors and the small numbers studied make a precise analysis of response rates for particular sites impossible. Single agent chemotherapy employing either hydroxyurea, cyclophosphamide, bleomycin, or methotrexate has been reported to produce objective remissions in 30-40 percent of patients with advanced epidermoid carcinoma of the head and neck region. Twelve patients with advanced head and neck cancer received infusions of 10 mg/day of dichloromethotrexate (DCM), an equipotent analog of methotrexate, which in contrast to methotrexate, is extensively metabolized by the liver. Nine patients had antitumor responses of 50 percent or greater. Leukopenia or thrombocytopenia was noted in only 3 of these patients, all of whom had pretreatment clinical evidence of liver disease presumed to be due to alcohol. This and other initial studies with DCM suggest that intra-arterial use of this compound may be of value.[251]

The National Wilms' Tumor Study is comparing the relative therapeutic effectiveness for adjuvant chemotherapy of actinomycin D (AMD) vincristine (VCR) and a combination of the two. The study will test the hypothesis that two drugs, each of which has a certain degree of effectiveness, can be combined to produce at least a summation of the therapeutic results achieved by either drug alone. This hypothesis presupposes that each drug has a different mechanism of action, that full dosage of each drug may be given, and that no cross resistance nor antagonism exists between the drugs.[252]

VCR and AMD are both effective in the treatment of children with Wilms' tumor. Current results suggest that, given optimal clinical circumstances, the use of both drugs in combination may be expected theoretically to achieve a 90 percent long-term survival rate in the primary cases, and a 60 percent retrieval rate in the metastatic cases.

Small cell carcinomas of the lung comprise 6-15 percent of all primary bronchogenic tumors. Patients with these highly malignant tumors have a median survival of about 2 months when diagnosed with extra-thoracic disease, and a five-year survival of less than 1 percent. Twenty-eight patients with small cell carcinoma of the lung were studied over a 17 month period for their response to combination chemotherapy and radiation therapy, frequency of bone

marrow metastases, and clinically evident hormonal syndromes.[253] Seventeen of 19 evaluable patients who received cyclic monthly courses of high-dose cyclophosphamide and vincristine had objective responses. Evaluation of procarbazine and methotrexate as a third adjuvant drug showed that only methotrexate (0.75-1.0 mg/kg) given on day 21 or days 21 and 28 proved beneficial with 6 of 14 patients responding and 8 of 14 showing no progression of disease. Radiation therapy to the primary tumor was effective in each of 3 dose schedules explored, but appeared optimal when 3200 rads given in 10 doses over 12 days (1401 rets) was administered at the completion of the second course of chemotherapy. Ten of 24 patients had bone marrow metastases at the time of the initial diagnosis and bone marrow aspiration should be used as a staging procedure. In a Phase II study,[254] 23 evaluable patients with documented small cell carcinoma of the lung were randomized among 3 schedules which included combined cyclophosphamide and vincristine, plus either procarbazine or methotrexate. Radiation therapy was given before, during, or after chemotherapy. Objective responses to chemotherapy were as follows: cyclophosphamide alone, 1 of 2 patients, cyclophosphamide and vincristine, 20 of 22 patients; procarbazine alone, 1 of 7 patients; methotrexate alone, 7 of 20 patients; methotrexate alone (objective response + no progression), 16 of 20 patients; and procarbazine alone (objective response + no progression), 2 of 7 patients. This technique, designed so that a third drug can be evaluated as a single agent, indicates that the combination of cyclophosphamide and vincristine provides a substantial improvement over cyclophosphamide alone; also methotrexate appears to be of value in this disease while procarbazine probably is not.

A clinical study of 32 evaluable patients with primary lung tumors, or inoperable or metastatic solid tumors were treated with vincristine and bleomycin. Vincristine was given at a dose of 1.0 mg to patients under 50 yrs. and 0.75 mg to patients 50 yrs. and older, as a rapid i.v. injection twice weekly. Six hours after each dose of vincristine, 30 mg of bleomycin was given, usually i.v. to 50 ml of a 5 percent dextrose solution. The twice-weekly doses were continued for a total of 6 weeks.[255] Of these patients, 9 had a partial response. They included 4 of 15 patients with lung cancer; 3 of 9 with head and neck cancer; and one each with testicular cancer and cloacogenic carcinoma. The response rate achieved with vincristine and bleomycin for lung cancer, compares favorably with the response rate obtained with bleomycin alone. A combination regimen of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), vincristine, methotrexate, and 5-fluorouracil was given to 43 patients with various advanced solid tumors. Thirty patients had an adequate trial for antitumor effect. The regimen was generally well tolerated and could be routinely administered on an outpatient basis. There were seven good responses and six fair responses in a variety of solid tumors. The best responses occurred in patients with pulmonary metastases from squamous carcinoma of the cervix (four of five responded), and in anaplastic thyroid cancer (one of one responded).[256]

Pleural effusion commonly complicates the course of malignant diseases. Not infrequently, effusions may be the first sign of metastases. Intracavitary quinacrine controls effusions by producing an inflammatory reaction at the serosal surface. The adhesive pleuritis obliterates the pleural space,

preventing the reaccumulation of fluid. A recent report details the clinical experience with quinacrine and tube thoracostomy in 39 patients with malignant diseases complicated by pleural effusions.[257] The response rate in 12 of these patients given intracavitary quinacrine in small daily doses was 67 percent. In the remaining 27 patients, quinacrine administered as a single dose through a thorocostomy tube, achieved a 70 percent response rate. There was no significant difference in response whether lung metastases were observed or not. Serious acute toxicity, including hypotension and hallucinations, probably increases with single dose therapy. Response rates of patients treated with quinacrine and thorocostomy indicated no advantage to exceeding 800 mg of quinacrine as a single dose. Further exploration of lower single doses of 600 mg or less with tube thoracostomy will be pursued. If clinical efficacy can be maintained at lower dose levels the frequency and degree of toxicity may well be less.

Adriamycin (NSC-123127) and bleomycin (NSC-125066) are new cytotoxic antibiotics each having antitumor activity in a wide spectrum of human neoplasms. Treatment with ADM and BLEO, alone and in combination has been evaluated in 56 patients with a variety of advanced stage gynecologic cancers.[258] ADM has a high degree of antitumor activity against uterine leiomyosarcoma and stromal sarcoma, and some of the unusual ovarian cancers including ovarian teratoma. ADM was also active and gave clinically worthwhile responses against squamous cell carcinoma of the cervix and vagina. BLEO has been found to have a range of antitumor activity which includes lymphomas and some other solid tumors. The combination of ADM plus BLEO appeared to show no enhancement of the effect achieved by ADM alone. There were no objective responses in patients with squamous cell carcinoma of the cervix treated with BLEO alone. The usual toxic manifestations of ADM and BLEO were observed, and there appeared to be no potentiation of the toxicity of each agent when used in combination. It is concluded that ADM is valuable for treating certain gynecologic cancers which are usually refractory to other chemotherapeutic agents. Further investigations of its use alone and in combination with other drugs are indicated.

A randomized study of 258 patients has demonstrated the superior efficacy of chlorambucil and prednisone combined as secondary therapy for advanced female breast cancer.[259] This combination therapy achieved an objective rate of 20.7 percent as compared with 11.8 percent for hydrocortisone with triiodothyronine, and 9.8 percent for chlorambucil alone.

IMMUNOTHERAPY

The finding of tumor-specific or tumor-associated antigens on human tumor cells and of cell-mediated and humoral immune responses to these antigens has stimulated clinical investigation in which various immunologic techniques have been applied to the prevention and treatment of human cancer. Immunotherapy is of current interest because in some tumor systems conventional therapy can reduce the tumor burden to the level at which immunotherapy in experimental systems becomes effective.

Most of the clinical studies initiated so far have been based on non-specific stimulation of cellular immune reactivity. The agent most commonly employed has been Bacillus Calmette-Guerin (BCG), which is known to be a potent immunologic stimulant, and is effective in some animal tumor systems. In one report[260] 28 patients with acute myeloblastic leukemia in whom remissions had been induced with combination chemotherapy, were randomized for prolongation of remission between BCG combined with methotrexate (MTX), or MTX alone. The median duration of remission (20.4 weeks) in those 14 patients receiving BCG was significantly longer than the 9.7 weeks for the 14 patients in the MTX group.

It has been demonstrated that survival with malignant lymphoma or chronic leukemia correlates with the status of the patients' cellular immune reactivity. The effects of BCG on delayed hypersensitivity responses were determined in a group of patients in various stages of disease.[261] BCG vaccination stimulated cellular immune responses of patients with well-controlled disease, regardless of stage, and repeated vaccination with BCG-cell mixtures resulted in cumulative increases of both delayed hypersensitivity to antigens of the vaccine mixtures and general stimulation of this class of immune responses. Patients with uncontrolled, actively progressing disease showed little immunologic response to BCG vaccination.

An exploratory trial[262] of immunologic stimulation in 15 patients with uncomplicated Philadelphia chromosome-positive leukemia has yielded favorable results. These patients were subjected to repeated vaccinations with BCG and allogeneic cultured cells. Survival appears to have been doubled by the addition of immunotherapy to antileukemic chemotherapy as compared with controls. It is not clear whether these favorable results reflect specific antileukemic immunization or nonspecific stimulation of cellular immune mechanisms.

Eight patients with Stage II and III melanomas were treated, after surgical excision of the tumor, with chemotherapy and non-specific immunotherapy with BCG. Five of these 8 patients had additional, although transient, clinical control of their malignant melanoma when inactivated mumps virus was added to the immunological stimulation sequence.[263]

Another preliminary report[264] of 13 patients with melanoma without clinically detectable disease but with high likelihood of relapse were treated with a Phase I immunotherapy program of live BCG given by scarification. Two dose levels were evaluated: high dose ($6x10^8$) live organisms, and low dose ($6x10^7$) live organisms. The data suggest that high-dose BCG may be associated with a heightened immune reactivity compared with low-dose BCG. This impression was strengthened by analysis of skin reactivity to established antigens and to the primary antigen, keyhole limpet hemocyanin (KLH). All relapse patients failed to develop hypersensitivity or antibody response to KLH. In contrast, remission patients all developed delayed hypersensitivity and a majority developed primary antibody response to KLH. Lymphocyte blastogenic responses to mitogens and antigens other than KLH were not significantly different between the relapse patients and the remission patients.

Another study[265] has shown clinical benefit in recurrent melanoma with lypophilized Tice-strain BCG compared with a liquid form of BCG (Pasteur strain). Eight of 9 patients treated with Tice BCG are alive and disease-free beyond one year. Sixteen of 19 with Pasteur BCG are still alive but only 8 are disease-free. There has been a significant decrease in relapse rate and an improvement in survival of patients treated with Tice BCG compared with surgery alone. High doses of Tice BCG induced a striking improvement in delayed hypersensitivity skin reactions.

In a group of 82 patients with solid neoplasms, BCG was well tolerated when given alone intradermally and when given alone or with a tumor-cell vaccine by the multiple puncture tine technique. However, the intratumor injection of BCG was associated with complications including malaise, influenza-like syndrome, fever, reversible liver dysfunction and jaundice.[266]

Active specific immunotherapy is another adjunctive approach to the treatment of cancer. Autoimmunization with non-irradiated acute leukemia cells not only increased the in vitro lymphocyte blastogenic reactivity to patients' own cells but also increased non-specific lymphocyte reactivity.[267] Six of 9 patients receiving 10^8 cells demonstrated a 100 percent or greater increase in response. In four responders, increased blastogenesis was noted only in autologous serum. Post-immunization serum may contain a factor responsible for the increased activity. Only one of four demonstrated increased response in autologous serum after immunization with 10^6 cells. Three patients studied 28-42 days after immunization showed a continued rise in the blastogenic response to leukemic cells.

The use of concentrates of tumor antigen may be of benefit for immune therapy. In a preliminary clinical study,[268] an extract of melanoma surface antigen has been used in an attempt to immunize the father of a patient with melanoma. Although no clinical benefit followed, an in vitro experiment demonstrated temporary sensitization of the father's lymphocytes to cultured tumor cells. This appeared to be sensitivity of the cell-mediated type since no antibody was demonstrated by cytotoxicity or immunofluorescence. The transience of the phenomenon suggests that the effect was due to tumor antigens. But the possibility of temporary sensitization to an HL-A antigen cannot be completely excluded.

Adoptive immunotherapy using viable sensitized lymphocytes has been of benefit to some patients with malignant disease. Therapeutic studies with cross-transplant, cross-transfusion were initiated in 64 patients with various neoplasms.[269] Minced fragments of viable tumor were implanted subcutaneously into matched pairs with similar tumors followed by cross-transfusions of plasma and white blood cells. In some cases, tumor cells grown in tissue culture, BCG, or transfer factor have been utilized. Three patients with melanoma had complete responses lasting 12, 46 and 51 months respectively; the latter two continue. Partial response occurred in six other patients. Three melanoma patients were immunized prophylactically after excision of metastases. They have developed antibodies against their tumor and have remained free of disease -- 1 for 2 years and 2 for 1 year. Results of this study suggest that immunotherapy is most effective when the patient is immunocompetent and has a minimal amount of residual tumor.

Preliminary investigations are under way to determine whether or not transfer factor (TF) therapy holds any hope for neoplastic disease. Recent studies have been concerned with the isolation and characterization of the active component of TF.[270] The chromatographic separation of TF from a tuberculin-sensitive donor resulted in isolation of a fraction which is biologically active as judged by its ability to transfer delayed hypersensitivity to tuberculin to non-immune recipients. Transfer of tumor immunity with TF was investigated in a patient with alveolar soft part sarcoma.[271] This patient and his identical twin brother were studied in terms of immunologic response to the patient's tumor homogenate. The lymphocytes from both twins underwent lymphoblastic transformation to tumor homogenate but only the healthy twin's lymphocytes released demonstrable migration inhibition factor (MIF) to the tumor preparation. TF was prepared from the healthy twin and administered to the tumor-bearing twin. A total dose of transfer factor equivalent to 45×10^8 lymphocytes given in three separate doses produced a persistently positive MIF assay in the patient. The tumor neither regressed nor progressed during the 6-month period after TF therapy.

"Immune" RNA extracted from sensitized lymphoid cells, is able to confer on nonsensitive patients with a variety of neoplastic diseases, including Hodgkin's disease, a state of specific sensitivity as demonstrated by the transfer of skin test response to PPD, Varidase, Monilia or mumps.[272] "Immune" RNA was antigen-specific and systemically active. Transfer was successful in a higher percentage of patients with localized disease, in remission, receiving no antitumor therapy and with positive skin test responses. In another study,[273] evidence has been presented that normal nonimmune rat lymphocytes can be converted to effector cells specifically cytotoxic to tumor cells, by incubation in vitro with syngeneic "immune" RNA extracted from lymphoid tissues of tumor-bearing rats. These results suggest the possibility that RNA extracted from lymphocytes of cancer patients may be effective in transferring immune responses to human tumors. Studies are in progress with RNA extracted from the peripheral blood lymphocytes of cancer patients with actively growing tumors, or from patients from whom tumors have recently been excised.

RADIOTHERAPY

Present radiotherapeutic efforts are in two broad areas: first the effective clinical application of presently available photon irradiation equipment, x- and gamma-ray sources, and the improvement of these applications whenever possible; second, the systematic exploration of the inherent characteristics of high linear-energy-transfer (high LET) radiations for more effective radiotherapy of localized, deep-seated neoplasms with minimum radiation to overlying or adjacent and surrounding normal tissues.

An interesting example in the first area is the use of a well-collimated, high-energy x-ray beam generated by a linear accelerator in the radiotherapy of patients with severe Graves' ophthalmopathy.[274] Anatomic, physiologic and immunologic concepts concerning the pathogenesis of the eye lesions constituted the rationale for the use of orbital irradiation to selectively ablate sensitized lymphocytes in the enlarged extra-ocular

muscles. Of 23 patients treated over a 4-year period beginning in March 1968, 15 experienced an excellent or good response, which occurred soon after the start of radiotherapy. No patient was made worse by this treatment. A favorable response to orbital irradiation was observed even in some patients who had previously responded poorly to systemic corticosteroid therapy.

Neutrons and charged particles (protons, electrons, negative pi mesons, and high- energy heavy nuclei) each produce different effects which, in comparison with conventional photon radiations, could provide significantly improved dose distribution, might largely overcome the radioresistance of anoxic tumor cells, and may also provide a more uniform effectiveness throughout the mitotic cycle of the cell.

Preliminary trials in which the biophysical factors pertinent to radiotherapy have been compared for low and high LET radiations,[275] and the therapeutic merits of various heavy particle radiation have been compared with conventional photon radiations,[276 277] have evolved three parameters of radiation quality: relative biological effectiveness (RBE), oxygen enhancement ratio (OER), and specific ionization or linear energy transfer (LET). These parameters are interrelated and no one of the three, by itself, is a satisfactory indicator of a given radiation.[278]

Fast neutron-emitting Californium-252 has been evaluated for interstitial and intracavitary use in cancer radiotherapy.[279] The findings are as follows: 1) Afterloading techniques are required to assume a reasonably geometric implant with Cf-252. 2) The clinical impression of 6.5 to 7.0 for the neutron RBE appears correct. 3) A number of patients with advanced disease have had local control of tumor indicating that Cf-252 is an effective interstitial source. 4) Intracavitary Cf-252 is feasible and may be easiest to standardize. A ratio of 0.45 μgr Cf-252 to 1.0 mg Ra-226 is suggested for gynecologic implants. 5) Increased precautions and care must be used in handling Cf-252 and with the patients being treated to prevent overexposure to health personnel. This must be weighed in assessing the usefulness of Cf-252. 6) Decay of Cf-252 ($t_{1/2}$=2.55y) may be a problem in maintaining adequate amounts for required intensities for gynecologic implants. A bank source may be needed for replenishments. 7) A controlled clinical trial will be needed to prove or disprove the greater effectiveness of Cf-252 over that of gamma emitters as Ra-226 or Ir-192.

Modern radiotherapeutic techniques for treating Hodgkin's disease and some lymphomas employ doses of 3,500 rads to 4,500 rads over extended fields extending beyond the overt foci of disease. In the process of irradiation of the major nodal sites on both sides of the diaphragm, a large portion of the bone marrow is exposed to considerable irradiation. The probability of ablation of a major portion of the bone marrow compartment exists with the current techniques.

A recent clinical study[280] demonstrated that ^{99m}Tc-S colloidal activity parallels ^{59}Fe and can be used for radioisotope scanning for mapping bone

marrow distribution and semiquantitative assessment of hematopoietic activity. The clinical evidence obtained from evaluating 27 patients with Hodgkin's disease using ^{99m}Tc-S colloid bone scanning techniques indicates that after 4,000 rads sequential segmental irradiation in patients with Hodgkin's disease there is evidence of significant suppression of bone marrow that occurs immediately following completion of radiotherapy and continues for a period of 1 or 2 years. Regeneration of bone marrow activity and expansion of bone marrow occurs at the end of about one year and continues to improve with time from the first to third post-irradiation year. Quantification of bone marrow activity is required to determine the true functional status in different bones after specific radiation doses at specific times. A maximum dose of 3,000 to 3,500 rads to occult foci in extended fields may be the desirable range to be certain of bone marrow regeneration and control of disease. Precise dosimetry is necessary to determine the dose to shielded remaining marrow sites. It appears that the dose-response data for bone marrow suppression following localized and segmental exposure need to be revised upwards for fractional irradiation.

A review[281] of 47 children with Stage I or II Hodgkin's disease treated by regional radiotherapy shows an 80 percent 5-year survival and a 67 percent 10-year survival. Twenty-seven of these patients were staged clinically and 20 were staged following acceptance of lymphography as a staging procedure for children. Celiotomy and splenectomy were not included routinely in the diagnostic studies until 1970. Local control was achieved uniformly. Patterns of new disease have been analyzed in relation to initial presentation and specific histology according to the method of Lukes and Butler.[281a] Possibilities for improving results, based on this retrospective study of clinical records, include combination treatment programs utilizing radiotherapy and chemotherapy.

Irregular fields are encountered in radiotherapy whenever radiation-sensitive structures are shielded from the main beam or when the field extends beyond the irregularly shaped patient body contours. "Mantle" fields employed in the treatment of Hodgkin's disease are extremely irregular in shape. The irregularity of the patient contour and blocking within the field, such as lung shields, makes approximation of the field area difficult.

A computer program[282] was developed embodying Clarkson's principle[282a] of separating the primary and scattered components of dose at a point in a phantom. This program is general for any shaped field and includes corrections for variations of exposure rate within the field, body contour, and non-nominal source-skin distances. Depth doses were calculated in several actual cases involving irregular fields such as mantle, split, L-shaped, and inverted Y fields. Data were examined to determine the degree of inhomogeneity in these fields. An approximation method used in the irregular field dosimetry was evaluated with regard to its accuracy and limitations. Comparison of depth dose determinations using both methods showed discrepancies of only 2 to 3 percent.

A new two-dimensional method for computer generation of dose distributions for external x- and gamma-ray sources has been described[283] in which emphasis

is on ease of implementation on small and moderate computers, and flexibility in application to computer-aided radiotherapy treatment planning. The method for calculation of the radiation fields does not require input or storage of tables, but depends on the natural parameters which describe beam geometry (field size, SSD, and diaphragm setting) and two parameters which describe change in scatter due to changing field size. Data are applicable to Co-60 and x-rays in the 4 to 31 MeV range. Fewer than 5,000 words of core, exclusive of I/O, and 2 to 3 seconds per field calculation time are needed.

The clinical data for a series of 51 patients treated with 92 separate lung fields for metastatic pulmonary disease between 1958 and 1971 were used to correlate the development of radiation pneumonitis with the variables known to influence its appearance.[284] Specifically, a formula for pulmonary damage related to dose, time, and number of fractions was developed on experimental radiation pneumonitis data in mice, and this formula was tested on patient data. The clinical treatment data are converted into nominal single doses (NSD) and a newly derived formula for estimated single doses (ED). In 28 of these 51 patients, dactinomycin was administered concurrently with radiation therapy, 550 to 6600 rads, delivered over periods of from 7 to 74 days with a 1-MeV x-ray unit, a cobalt-60 unit, or a 4 MeV linear accelerator. The 5 percent incidence level of pneumonitis without dactinomycin was found to be 770 rets (NSD) and 510 rets (ED). It was concluded that a safe treatment regimen for avoiding radiation pneumonitis is 1500 rads in 10 fractions with dactinomycin, and 2500 rads in 20 fractions without dactinomycin.

The megavoltage electron beam has several characteristics which permit ideal irradiation of the cervical lymphatics vasculature in head and neck cancers, and in treatment of cancer of the skin and lips: relatively high surface dose (calculated to be from 83 to 90 percent for energies from 6 to 12 MeV, and more than 90 percent for 15 to 18 MeV); the steep build-up to maximum dose; the sharp fall-off in dose beyond the Bragg peak; and selection of depth of the peak dose by varying the energy.

In one study,[285] 75 patients with head and neck cancers have received electron beam treatment to one side of the neck, and to both sides in 6 patients. Electron beam therapy has been given after radical neck dissection for extensive neck disease, or for recurrent disease in the neck occurring 2 to 26 months after radical neck dissection. Eleven of 31 patients receiving elective irradiation after neck dissection are alive with no evidence of disease up to 8 years after treatment. By comparison, in other clinical reports for patients who did not receive preoperative irradiation, a cervical recurrence rate of 54 percent has been found in the radically dissected neck of patients with positive lymph nodes, and a 71 percent recurrence rate in patients with lymph nodes positive at multiple levels. It is for the purpose of preventing this anticipated high rate of recurrence after neck dissection that radiation therapy, done ideally with the electron beam for one side, is given when many lymph nodes are positive or when tumor is found in connective tissue.

In another study,[286] 156 patients with squamous and basal-cell carcinomas of the skin and lip were treated with a 7 to 18 MeV electron beam. This is a selected series of patients, presenting varied and difficult problems in management. One-half of these patients had prior surgical treatment; many of the lesions had recurred several times, and many were bulky and extensively infiltrative. For the satisfactory treatment of superficial lesions about the eyelids and inner canthi, the nose, the lips, the skin of the neck, the ear, the hand, and the flank, it is essential to achieve sharp field definition and protection of normal structures of special functional importance. The electron beam is particularly useful since underlying normal structures are minimally damaged and adjacent structures are easily protected with thin sheets of lead. Primary control with electron beam treatment was 86 percent for all sites in 111 evaluable patients followed for 2 to 8.5 years. With surgical treatment of 15 patients who had persistence or recurrence of disease, 10 patients were salvaged with a final control rate of 96 percent and excellent cosmetic results.

While there is much cytogenetic information on the radiation response of human chromosomes in blood cells, from irradiated patients or cultures, there is an almost complete absence of information on radiation-induced mutation in human germ cells, so that in order to qualify risk in terms of mutation rate per locus per rad, it is necessary to extrapolate from experimental results obtained with other organisms. A re-examination of the data for radiation-induced forward mutation rates from various organisms ranging from bacteria to fungi, higher plants, insects and including mammals for each of which the amount of DNA per nucleus is known, shows that the mutation rates per locus per rad vary over a range of 1,000. Nonetheless, when each of these rates is adjusted for the amount of DNA per nucleus and thus normalized to a common biological baseline, the mutation rates then obtained are all essentially the same, varying by a factor of about three instead of 1,000. This remarkable consistency in normalized mutation rates in such a wide variety of organisms suggests that extrapolation directly from other organisms to humans may be done with confidence.[287]

The cyclotron facility at the Naval Research Laboratory (NRL) at Washington, D. C. is being utilized for a full-scale, statistically significant five-year program of controlled clinical trials of neutron radiation therapy. The Middle Atlantic Neutron Therapy Association (MANTA) has established schedules for each of four phases. Phase 1 is physical characterization of the fast neutron beam of the NRL cyclotron. Phase 2 consists of preliminary biological studies. Phase 3 includes facility modifications for optimized patient handling, a pilot study, and late neutron irradiation effects studies. Phase 4, scheduled to begin July 1975, is a five-year program of controlled clinical trials. Considerable progress has been made in facility modification, equipment development, measurement of beam properties, dosimetry inter-comparisons and biology experiments.[288] Work is reported to be proceeding on schedule.

A hospital-based negative pi-meson radiotherapy facility is now being designed for Stanford University Medical Center.[289] A 500 MeV electron beam from a superconducting linear electron accelerator will generate negative

pions, which will then be directed by superconducting magnets through a novel 60-sectored pion channel, making possible simultaneous multiport irradiation. The pion channel design permits the magnetic field to be varied in time, and the number of sectors transmitting pions can be preselected and adjusted during treatment, thus providing maximal flexibility in shaping the "star region" of the beam to the continuously changing x-y contours of the tumor while the patient, on a specially designed support assembly, is moved in the Z axis. It is expected that this accelerator can be housed in a space 100 feet in length by 20 feet in width. The combined facilities are estimated to make possible the radiotherapy of a minimum of 1,000 new patients per year.

The U.S. Atomic Energy Commission is constructing the Los Alamos Meson Physics Facility (LAMPF) to do research in basic nuclear physics. Because the potential of pions for radiotherapy was recognized some time ago, it was decided that a biomedical facility should be built in conjunction with the main LAMPF accelerator. The National Cancer Institute is sharing in the cost of this biomedical facility. This construction is Phase I of three phases in the program to evaluate pion radiotherapy. The central apparatus in the biomedical facility is the beam line which is used to collect, purify and focus ions produced by the main LAMPF proton beam. Phase II provides for pre-clinical experiments and the third phase is oriented toward clinical trials. The general objective of Phase II is to provide information crucial for beginning human biology and a clinical trial. It contains three elements: (1) beam operation, control, diagnostics and dosimetry; (2) cell system base lines and cellular radiobiology; and (3) animal (normal and tumor) tissue base lines and tissue radiobiology.[289a]

A report[290] describes PIPLAN, a treatment-planning computer code for use in planning pion radiation therapy treatments. This report includes an introduction to the code, instructions for its use and an illustrative example.

SUPPORTIVE THERAPY

Patients with acute leukemia undergoing chemotherapy have a high risk of infections, particularly by Gram-negative bacilli, which frequently are resistant to antibiotic therapy. Because the skin may harbor potentially pathogenic organisms, and the skin is a common site of infection in leukemic patients, the effects of bathing with P-300, a new bacteriostatic soap, were studied in 32 patients in protected environment units.[291] There was a major reduction in the concentration of bacterial colony-forming units on every body site following bathing with P-300. Sixty percent of the 186 strains of organisms cultured were eliminated within two weeks. Thirty-two of 45 potentially pathogenic organisms were eliminated, including all strains of Staphylococcus aureus.

A decade of studies on the epidemiology, natural history and treatment of Pseudomonas aeruginosa infections in cancer patients is summarized in a recent report.[292] Ten years ago a neutropenic patient with Pseudomonas infection almost always died. Today, because of advances in the management

of such infections, the vast majority recover and many become asymptomatic within 24 to 48 hours. Polymixin B and colistin were the first antibiotics introduced which had antipseudomonal activity. These were followed by gentamycin, and carbenicillin; the latter is effective even in patients with severe neutropenia. Several other penicillins have been synthesized which have antipseudomonal activity. The first of these was 6-(D-α sulfoamino) penicillin (BL-P 1462). Ticarcillin (BRL-2288) is another new penicillin that is more active than carbenicillin or BL-P 1462 in vitro against P. aeruginosa. Of a series of (α-S-substituted ureido) penicillins, one derivative, 6(D-α guanylureido) penicillin (BL-P 1654) has been selected for clinical evaluation.

Several new aminoglycoside antibiotics with antipseudomonal activity are currently under investigation. In a comparative study of 100 isolates, tobramycin at a concentration of 0.10 μg/ml inhibited 80 percent, whereas gentamycin inhibited only 4 percent. BB-K8, a derivative of kanamycin sulfate, in addition to activity against P. aeruginosa, has broad-spectrum activity against Gram-negative bacilli which are resistant to other aminoglycosides. Butirosin has broad-spectrum activity against Gram-negative bacilli in addition to antipseudomonal activity, and against clinical isolates resistant to gentamycin. Clinical studies of this drug have not yet been done.

New chemotherapeutic approaches have improved considerably the survival of patients with many malignancies including acute leukemia, soft tissue sarcomas, and breast carcinoma. The ability to control Pseudomonas infections has played a contributing role in this success.

The patient with leukemia undergoing the initial induction phase of therapy represents a transition from a host compromised solely from the malignancy to one with beginning resolution of the primary disease but with the added burdens of immunosuppressive therapy. Delineation of the pattern of infections from the untreated leukemic state through induction of remission should give some insight into the effects of malignancy vs. the effects of chemotherapy on the incidence and types of acquired infectious diseases. Also, it is important to know what effect infection has on the induction of remission as well as on the subsequent course of leukemia.

The effects of infectious diseases during the transition from untreated acute lymphocytic leukemia to induction of remission were studied in 100 children enrolled consecutively in a standardized protocol for chemotherapy.[293] During the initial 6 weeks of therapy, 30 patients had no evidence of infection; 19 had episodes of fever without other evidence of infection, and 51 had one or more episodes of infectious diseases. Most of the infections and febrile episodes occurred prior to and during the first week of therapy. Bacterial sepsis or meningitis and systemic candidiasis accounted for the serious infections. The incidence was inversely related to the absolute neutrophil count. Immunoglobulin values were normal.

The data suggest that infection during initial therapy does not adversely affect chances for remission induction. Vigorous measures should therefore

be taken to control infection during remission induction because the child who recovers from infection has an excellent chance of achieving remission and of continuing in remission for a long time. With total therapy, a 55 percent chance for 5-year leukemia-free survival can be expected.

Fever continues to be a major diagnostic and therapeutic problem, especially in cancer patients with neutropenia since these patients are unable to react to infections with a normal inflammatory response and their infections often disseminate rapidly. For this reason many neutropenic patients will die unless they are treated promptly with effective antibiotics. Consequently broad-spectrum antibiotic therapy should be instituted promptly even if the cause of fever cannot be immediately established.

Since the management of fever of unknown origin in cancer patients with neutropenia has not been systematically investigated, a study was designed to determine whether antibiotics should be continued if a causative organism has still not been identified after 4 days.[294] This study included 81 febrile episodes in 75 patients. In the preliminary phase, neutropenic patients with temperatures of at least 101°F, persisting for more than 4 hours after transfusion, were started on combination carbenicillin and cephalothin and continued for 4 days. Patients in whom infection was documented within 4 days were treated with appropriate antibiotics and were not further included in this study. After the four days of combined carbenicillin and cephalothin therapy, separate randomizations to continue or discontinue antibiotic therapy were made for those patients who had become afebrile, and for those who had persistent fever. The duration of additional therapy was 10 days, or 5 days after becoming afebrile, whichever was longer. Whenever infection with an organism not sensitive to either carbenicillin or cephalin was documented, the regimen was modified to include the appropriate antibiotic.

Analysis of the data obtained in this study indicate that antibiotics should be continued in patients whose fever responds because about 20 percent of these patients are infected. Patients whose fever continues have about a 40 percent probability of being infected but require additional antibiotics. For patients not responding after the initial 4 days of therapy, the addition of gentamicin is suggested.

PROGNOSIS

A formal evaluation of the Rye classification of Hodgkin's disease was done with special reference to the histologic data and the predictability of prognosis.[295] It was first necessary to establish the extent to which any one histologic feature evaluated carried information about the others. The relationship of prognosis to the histologic parameters and classification was then established. The analysis was completed by establishing which histologic features added further prognostic information to the Rye classification.

The Rye classification of Hodgkin's disease was found to furnish more prognostic information than any estimates of individual cell frequencies. However, analysis of information obtained by cross-classification between the

Rye subtypes of Hodgkin's disease and cellular composition showed that knowledge of the lymphocyte frequency significantly improved the predictability of prognosis in the nodular sclerosis and mixed cellularity types. Mean survival in both forms was directly proportional to the lymphocyte frequency in the pre-treatment biopsies. Malignant and benign-appearing histiocytes adversely affected prognosis when the levels of malignant histiocytes were relatively low. Sternberg-Reed cell had no effect, independent of malignant histiocytes, and eosinophils and plasma cells had no prognostic value.

An initial retrospective study was done to identify factors affecting survival in patients with cancer of the breast.[296] Fifty-eight breast cancer patients with varying survival times were studied by a matched pair analysis for 17 variables. Pairs were matched for menstrual status at time of surgery, age within 5 years, and the longest survival time with the shortest survival time. Sign and rank tests were used for analysis. The following differences gave p values of about 0.1 or less. In the postmenopausal group (20 pairs), non-survivors showed a longer interval between onset of symptoms and the first treatment,a larger number of positive nodes at the time of surgery and a poorer histological grade of tumor than survivors. In the premenopausal group (9 pairs) the preoperative white blood cell and monocyte counts were lower, while preoperative platelet counts were higher in non-survivors than in survivors. The first three findings were previously known and confirmed by this method. The last three factors have not been recognized previously and may relate to the immune status of the host. The most significant difference was the lower monocyte count in non-survivors. Recent studies suggest that macrophages have a cytotoxic effect on transformed cells. The reduced monocyte count may reflect a reduced tissue macrophage level and therefore a reduced capability of the patient to respond to her tumor.

A mathematical model has been developed and used to estimate the relative importance of presenting signs and symptoms in prediction of survival of patients with Stages III and IV carcinoma of the prostate gland.[297] The choice of an appropriate model involves several considerations: simplicity in concept, mathematical tractability, adequacy in fitting the data, and ease of interpretation in biologic terms. In this study, the mathematical analysis was based on the clinical data from 1824 patients with prostatic cancer in a clinical trial conducted by the Veterans Administration Cooperative Urological Research Group. For this patient group 72,174 actual patient-months of follow-up information was used. The clinical information consisted of eleven "concomitant variables". To determine which concomitant variables were most useful in prediction of survival, death without regard to cause was used as the end point. The variables, in the order of the magnitude of their regression coefficients were: totally bedridden; partially bedridden; acid phosphatase over 5.0 (King-Armstrong Units); force of mortality; metastasis; acid phosphatase 2.1-5.0; hemoglobin 12g/100 ml; ureteral dilation; acid phosphatase 1.1-2.0; weight under 130 lbs; age 70 or over; pain due to cancer. Survival predictions based on this experimental model agreed remarkably well with observed survival rates.

A review of 156 children with a diagnosis of Wilms' tumor considered the clinical factors that relate to prognosis.[298] A T-N-M staging system, based

on primary tumor extent at laparotomy, nodal involvement, and metastatic spread, was devised to relate disease extent to prognosis. Large primary tumor size and major extrarenal vascular involvement did not affect the prognosis within a given stage. Tumor spill noted at operation requires subsequent whole abdominal irradiation in adequate doses to prevent local abdominal recurrence. A high correlation of vascular involvement with pulmonary metastases was found. Pulmonary metastases with or without hilar and/or mediastinal disease predominated as the initial site of relapse, being the sole initial metastatic site in 63 percent of the cases. Control of metastases was obtained in about half of these cases by aggressive irradiation and chemotherapy. No demonstrable effect of age at presentation on subsequent prognosis could be identified.

In children, perhaps more than in any other patient group, considerations of the risks of treatment must be weighed against potential benefits. Children cured of their tumor will survive many years to suffer the effects of their treatment. Nevertheless, it is believed that the potential of disease cure in more than 70 percent of patients with Wilms' tumor utilizing surgery, radiation therapy, and chemotherapy justifies some complications. Since the risks of serious complications appear greatest with relapse in a previously treated area which is then retreated, it appears clear that adequate initial therapy is most important.

In dealing with individual patients with Wilms' tumor it is highly desirable to give a prognostic opinion with some degree of accuracy. Furthermore, the appropriate therapeutic effort for each case should be individualized for optimum results. A retrospective analysis of 49 cases of Wilms' tumor indicated that the most important prognostic factors were the anatomical stage of the tumor and pathologic findings such as invasion of the renal capsule, the renal vein or inferior vena cava, and metastases to the regional lymph nodes or to other organs. Stage I lesions had a very good prognosis (85 percent 3-year survival NED), even without high-dose irradiation or prolonged chemotherapy. Triple therapy, involving nephrectomy, irradiation and prolonged chemotherapy yielded a 55 percent survival rate in patients with Stage II tumors and 30 percent with Stage IV (patients with pulmonary metastases). Stage III tumors showed an unsatisfactory response to this comprehensive approach. Patients with bilateral tumors are potentially curable if aggressively treated. From this study it can be concluded that: 1. thorough analysis of the clinical and pathologic characteristics of the tumor should be made in every case and management should be individualized to avoid excessive treatment in early lesions with favorable prognosis, and 2. carefully administered postoperative irradiation and prolonged chemotherapy may improve the poor results in extensive lesions, even in the presence of distant metastases, with relatively minor sequelae in the surviving children. The late effects of vigorous and prolonged chemotherapy on the bone marrow, the immunologic surveillance system, and the germinal cells of the gonads as well as the repercussion on normal growth of these children remain to be evaluated.[299]

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